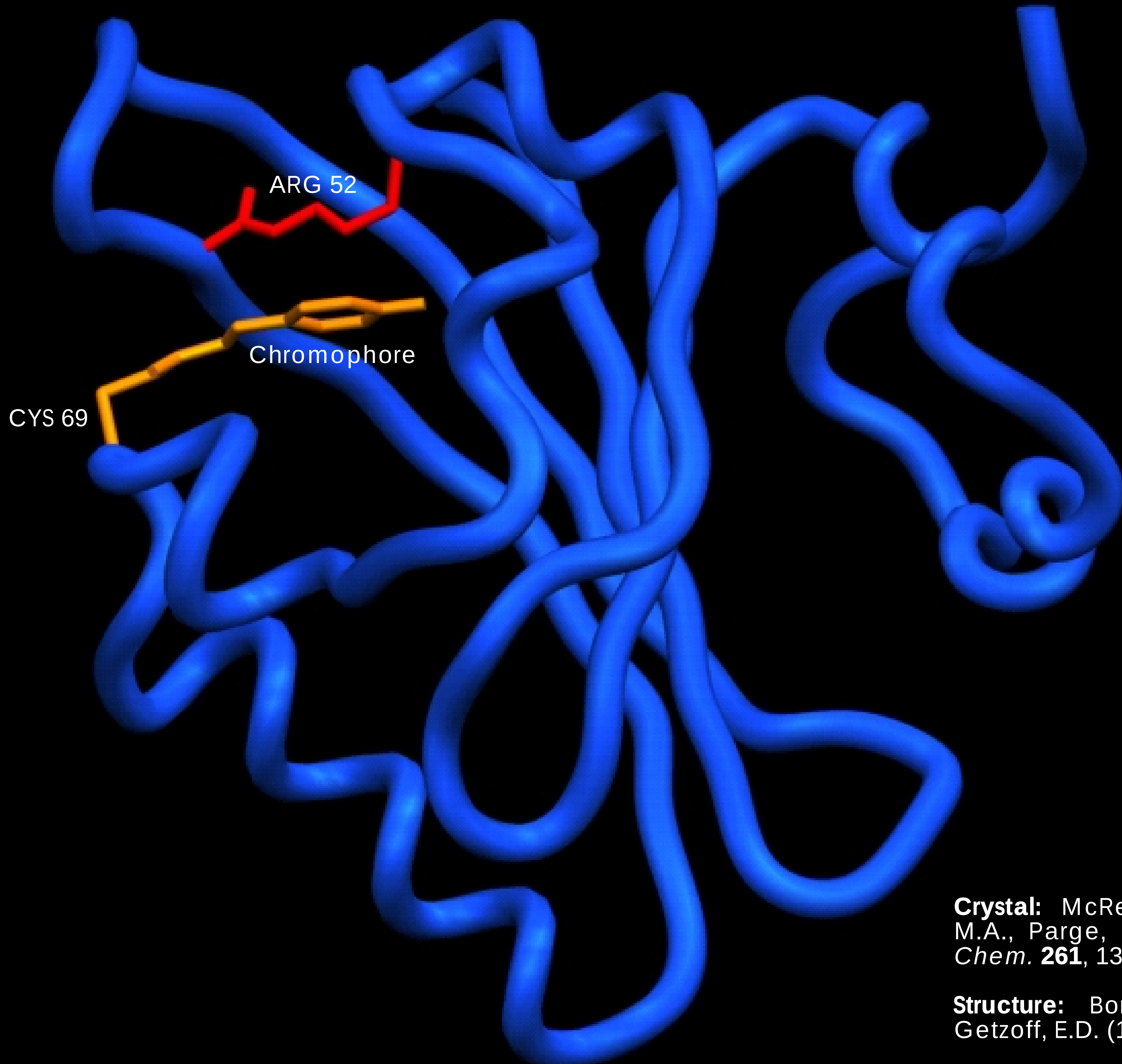
& Monochromatic

Laue Diffraction Techniques for Time-resolved Crystallography

Zhong Ren
CARS, The University of Chicago

Outline

- Time-resolved crystallography
- Laue diffraction
- Large-angle rotation geometry (LARG)

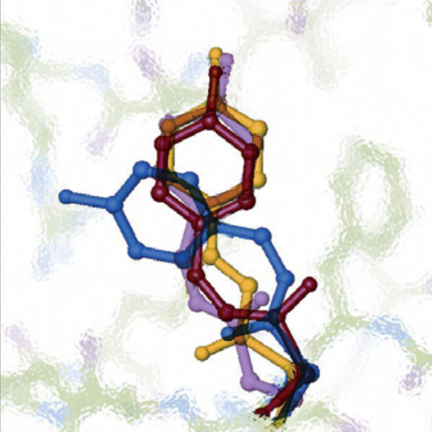


Photoactive Yellow Protein

Photoactive yellow protein (PYP), isolated from purple sulfur bacterium *Ectothiorhodospira halophila*, is a water−soluble 14 kDa photoreceptor protein. The crystal structure at 1.4 Å resolution shows its α/β fold similar to that of eukaryotic proteins involved in signal transduction.

Crystal: McRee, D.E., Meyer, T.E., Cusanovich, M.A., Parge, H.E. & Getzoff, E.D. (1986). *J. Biol. Chem.* **261**, 13850−1.

Structure: Borgstahl, G.E.O., Williams, D.R. & Getzoff, E.D. (1995). *Biochemistry*, **34**, 6278−87.



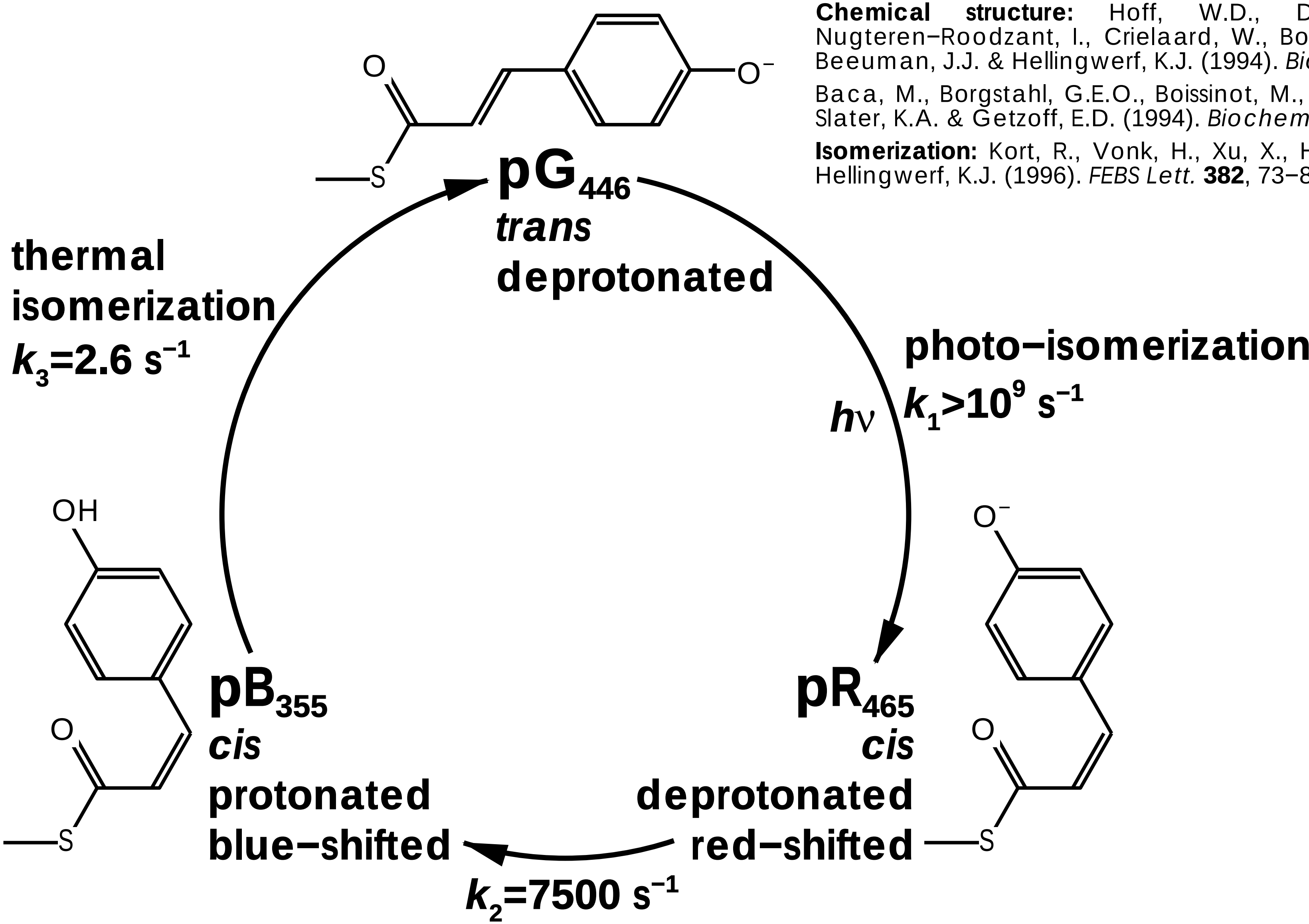
Photocycle: Meyer, T.E., Yakali, E., Cusanovich, M.A. & Tollin, G. (1987). *Biochemistry* **26**, 418−23.

Hoff, W.D., Van Stokkum, I.H.M., Van Ramesdonk, H.J., Van Brederode, M.E., Brouwer, A.M., Fitch, J.E., Meyer, T.E., Van Grondelle, R. & Hellingwerf, K.J. (1994). *Biophys. J.* **67**, 1691−705.

Chemical structure: Hoff, W.D., Dux, P., Devreese, B., Nugteren−Roodzant, I., Crielaard, W., Boelens, R., Kaptein, R., Van Beeuman, J.J. & Hellingwerf, K.J. (1994). *Biochemistry* **33**, 13959−62.

Baca, M., Borgstahl, G.E.O., Boissinot, M., Burke, P.M., Williams, W.R., Slater, K.A. & Getzoff, E.D. (1994). *Biochemistry* **33**, 14369−77.

Isomerization: Kort, R., Vonk, H., Xu, X., Hoff, W.D., Crielaard, W. & Hellingwerf, K.J. (1996). *FEBS Lett.* **382**, 73−8.



$$P_1 \xrightarrow{k_1} P_2 \xrightarrow{k_2} P_3 \xrightarrow{k_3} P_4$$

$$c_1'(t) = -k_1 c_1(t),\ c_1(0) = 1$$

$$c_2'(t) = k_1 c_1(t) - k_2 c_2(t),\ c_2(0) = 0$$

$$c_3'(t) = k_2 c_2(t) - k_3 c_3(t),\ c_3(0) = 0$$

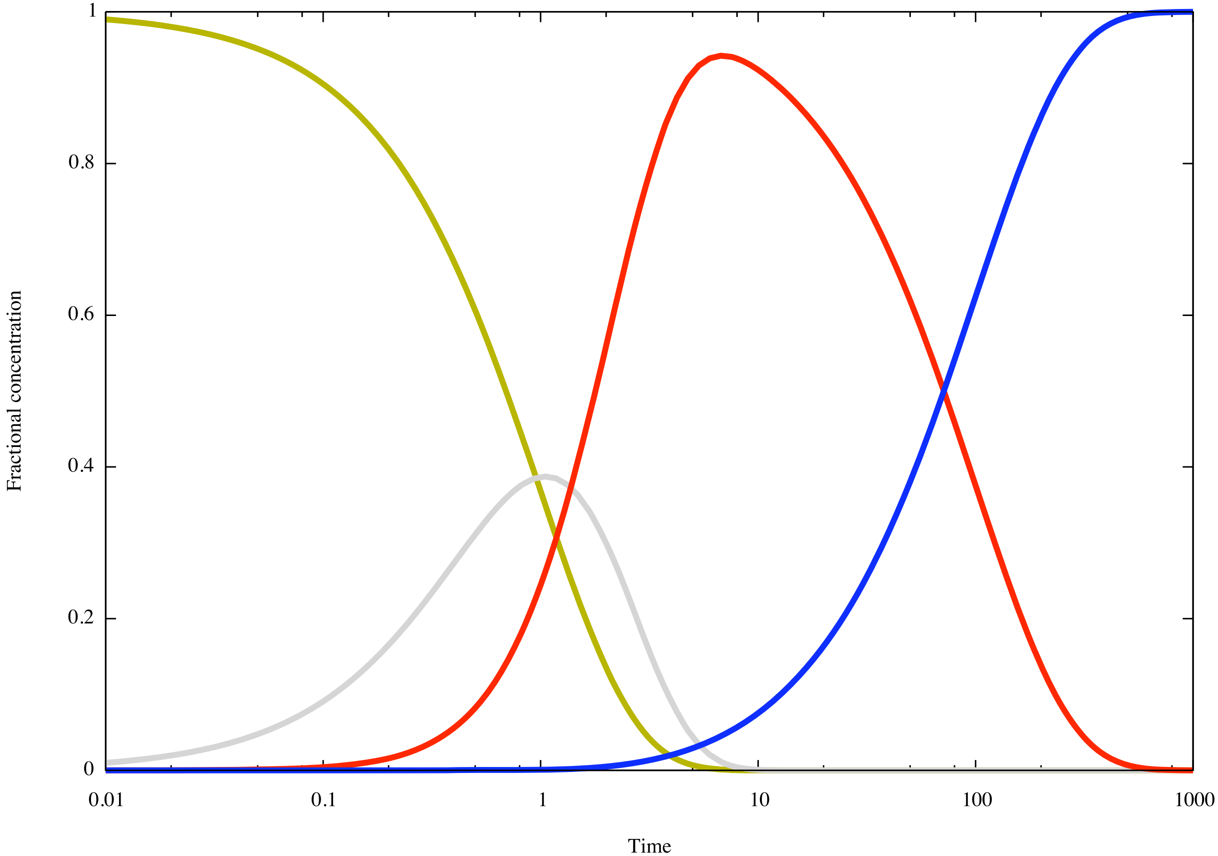
$$c_4'(t) = k_3 c_3(t),\ c_4(0) = 0$$

$$c_1(t) = e^{-t}$$

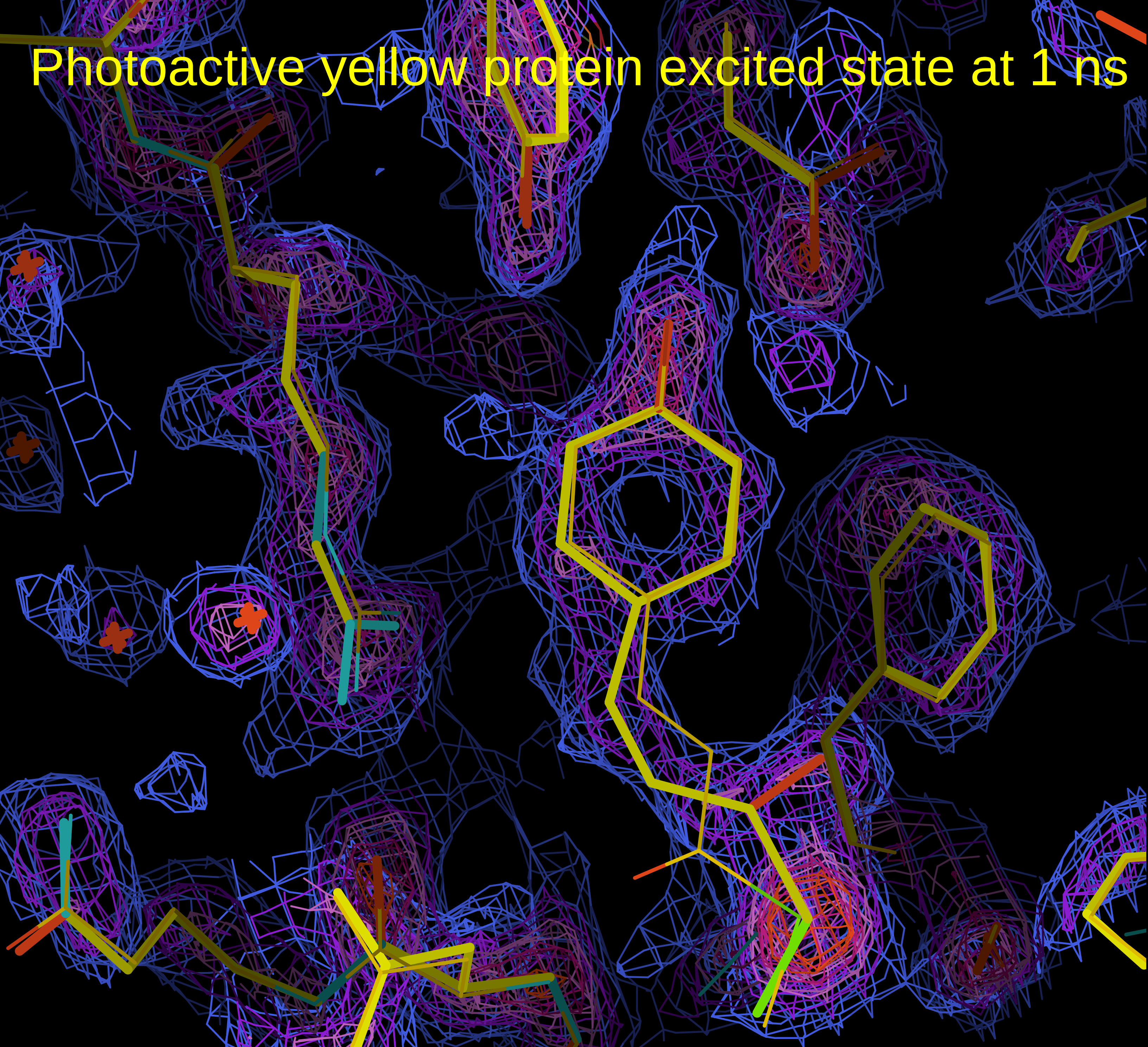
$$c_2(t) = \frac{e^{-t} - e^{-k_2 t}}{-1 + k_2}$$

$$c_3(t) = -\frac{e^{-k_3 t} k_2 \left(1 - k_2 + e^{(-1+k_3)t}(k_2 - k_3) + e^{(-k_2+k_3)t}(-1 + k_3)\right)}{(-1 + k_2)(-1 + k_3)(-k_2 + k_3)}$$

$$c_3(t) = 1 - c_1(t) - c_2(t) - c_3(t)$$


Fractional concentration
Time
0
0.2
0.4
0.6
0.8
1
0.01
0.1
1
10
100
1000

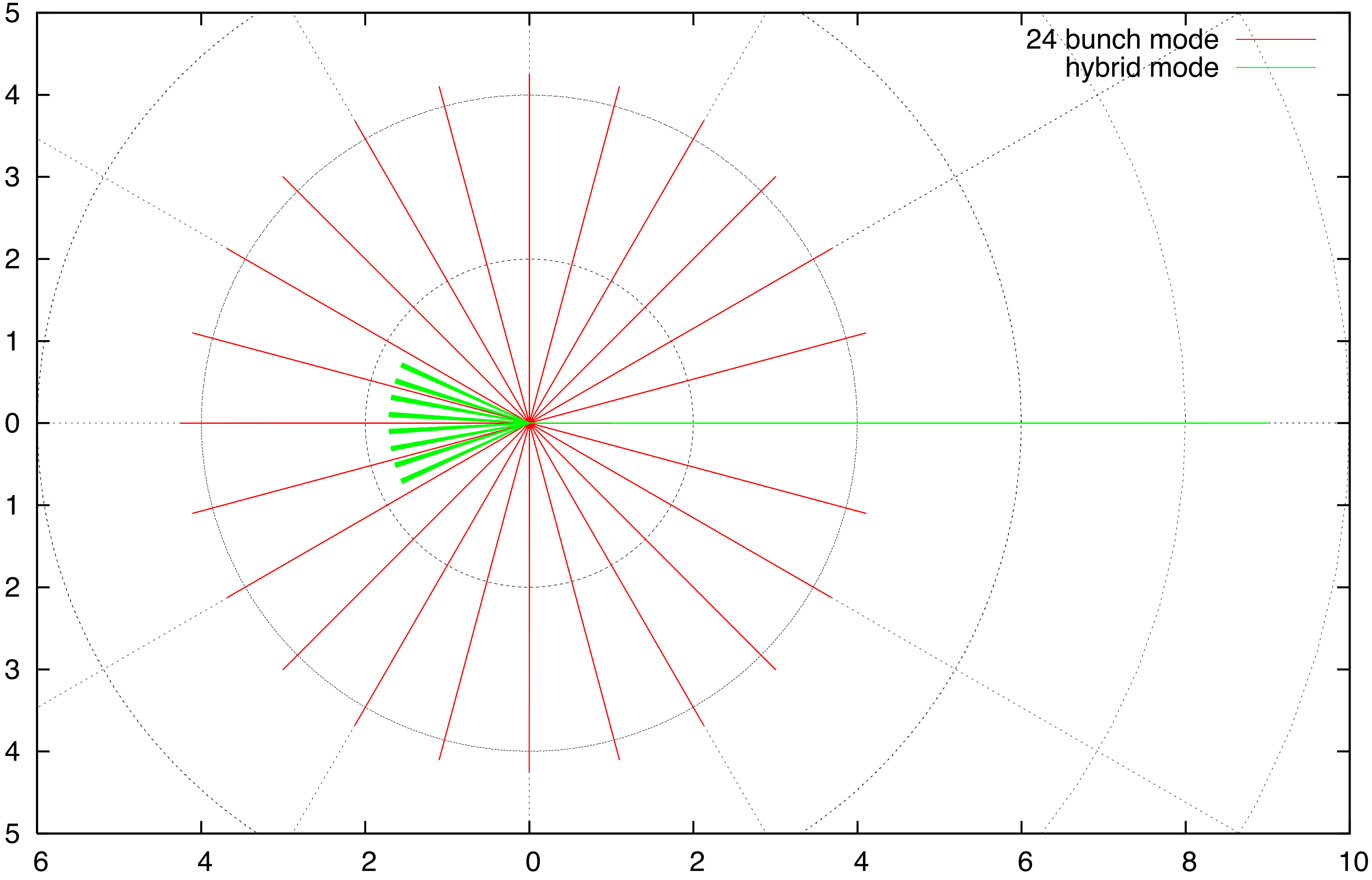
animation


Photoactive yellow protein excited state at 1 ns

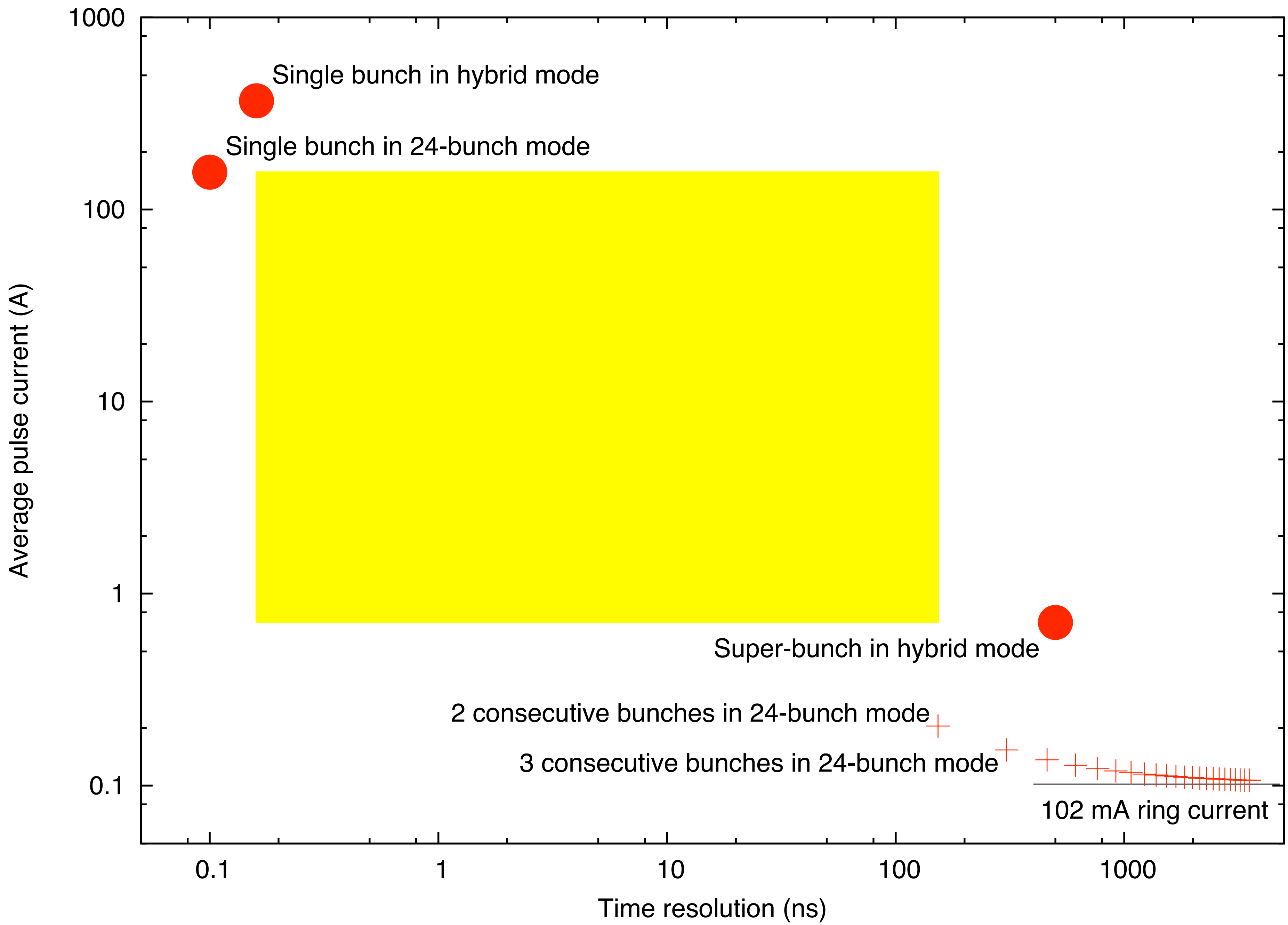
Possible ways to vary θ and/or λ in Bragg equation $2d\sin\theta = \lambda$ to measure complete integrated intensity

Crystal motion 0 – motionless 1 – with motion	X-ray wavelength 0 – fixed monochromatic 1 – polychromatic or scanning monochromatic	Incident angle 0 – highly collimated 1 – variable, e.g., fan, cone, angular sweep	Experiment type
0	0	0	Incomplete integrated intensity
1	0	0	Monochromatic oscillation
0	1 (polychromatic)	0	White (pink) beam Laue
0	1 (scanning monochromatic)	0	Monochromatic Laue
0	0	1	FEL/4th generation source
0	1	1	Smeared Laue
1	0	1	Highly focused mono
1	1	0	Laue oscillation
1	1	1	Mess

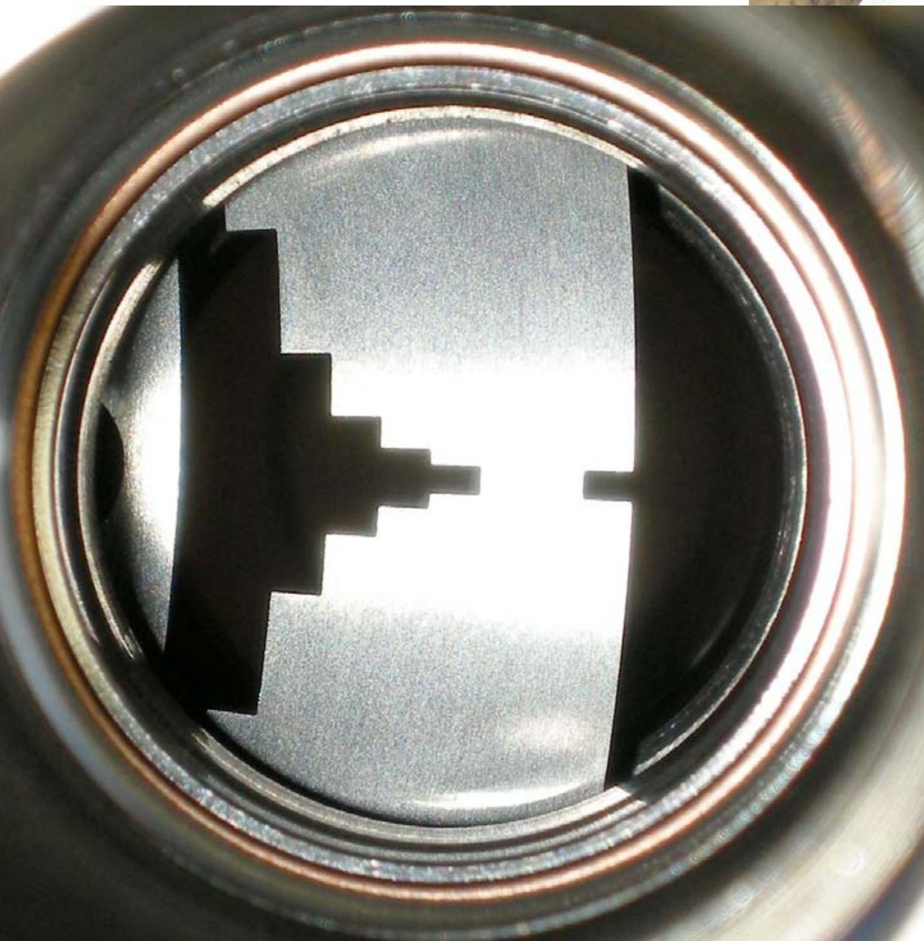
Time-resolved	Fast	Laue diffraction: polychromatic motionless
Static	Slow	Conventional technique: monochromatic oscillation/rotation

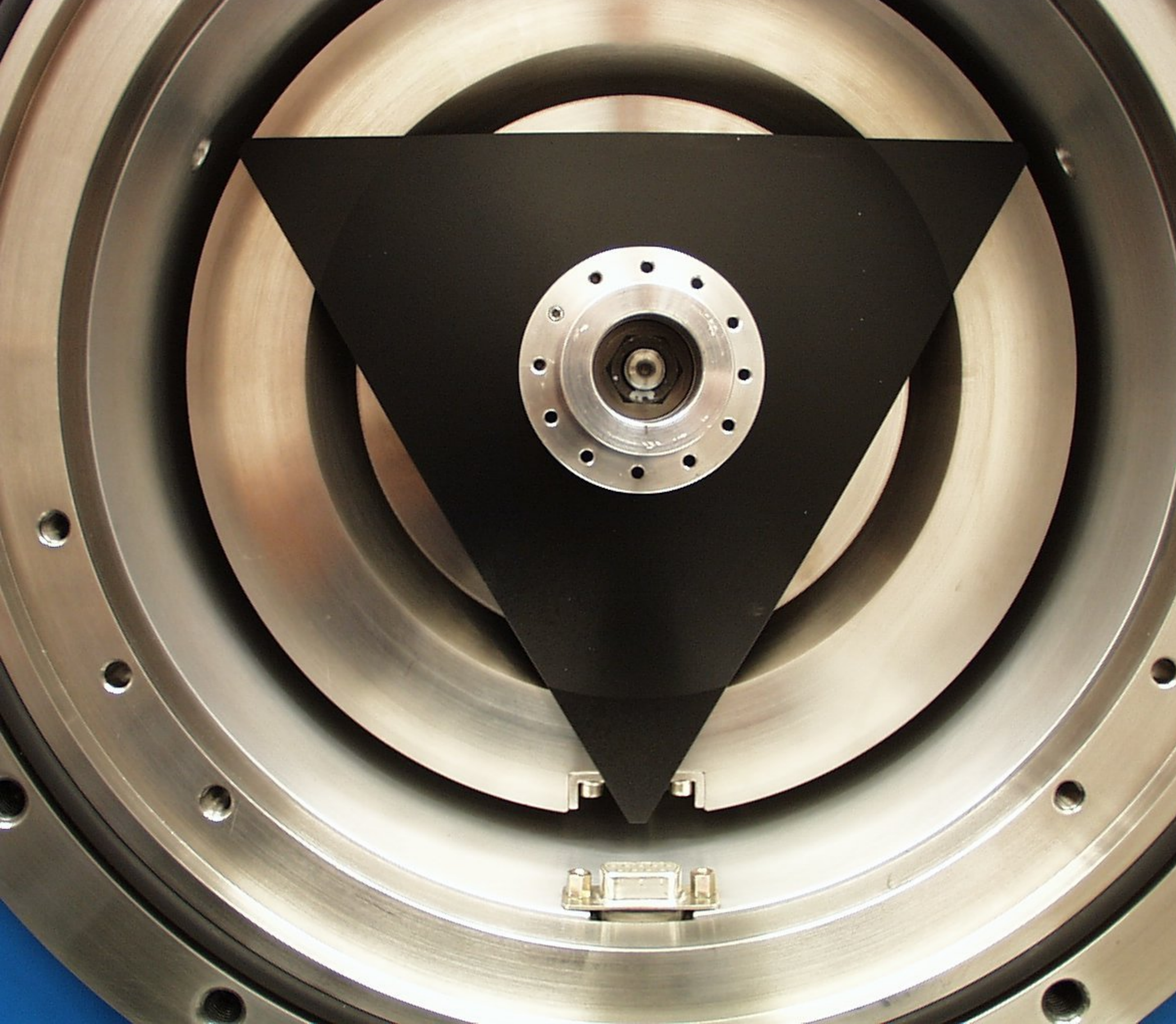


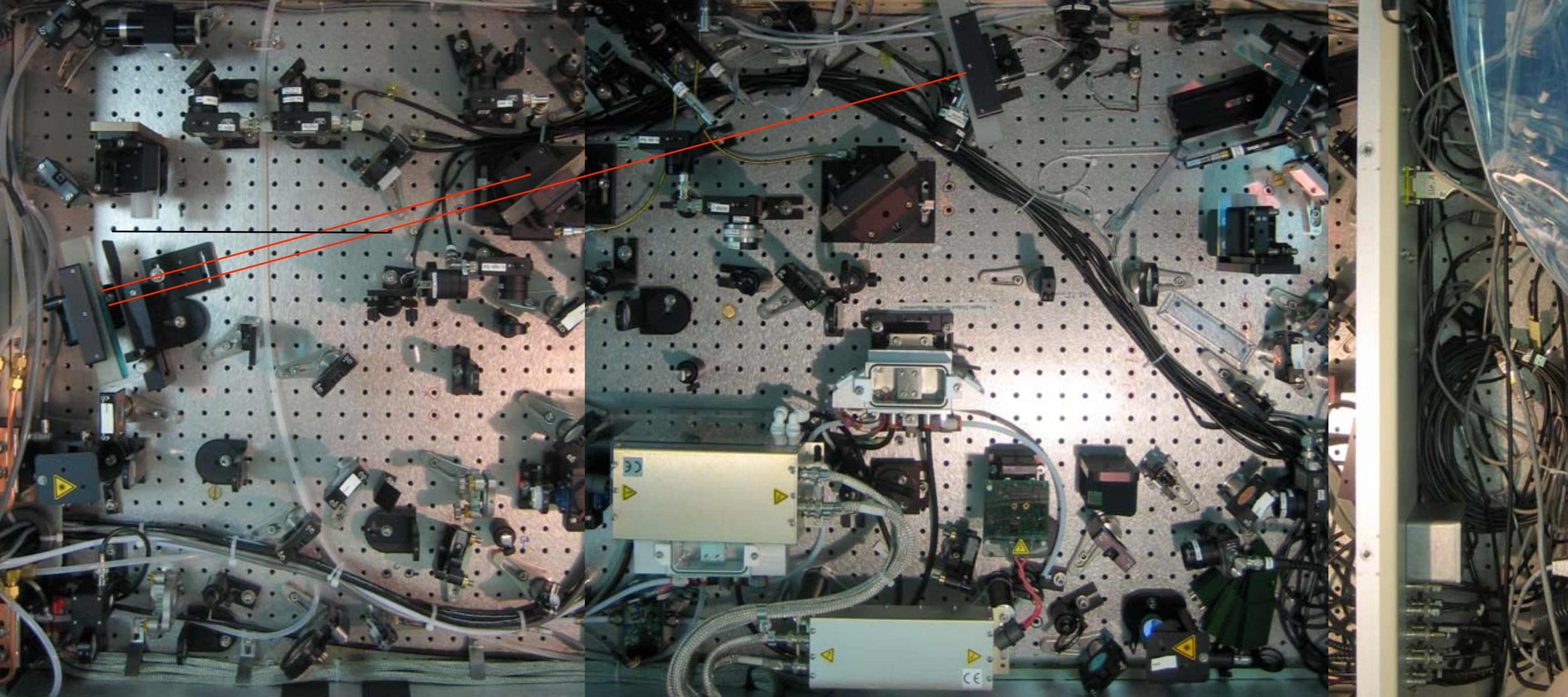
24 bunch mode
hybrid mode
5
4
3
2
1
0
1
2
3
4
5
6
4
2
0
2
4
6
8
10

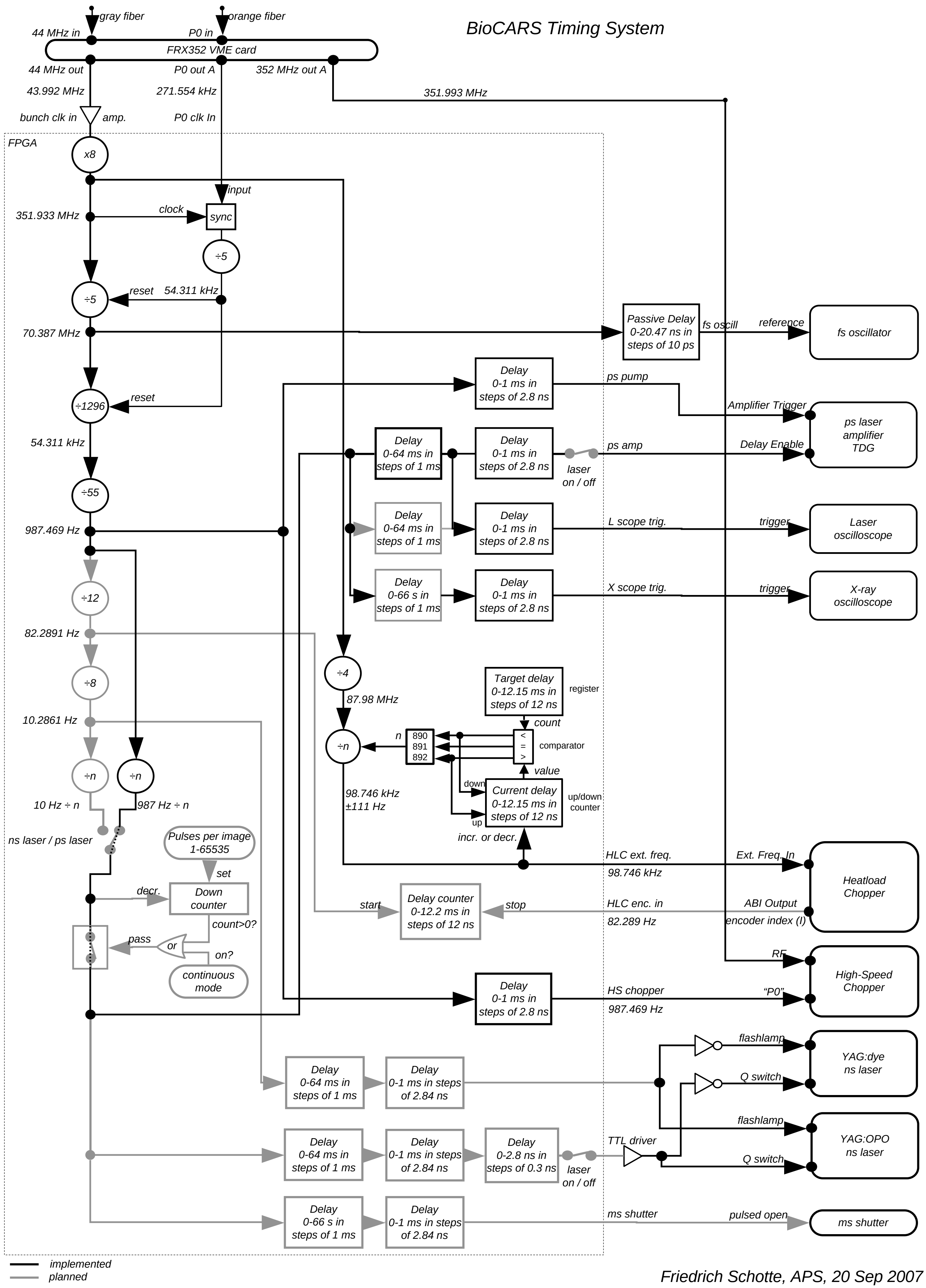


1000
100
10
1
0.1
Average pulse current (A)
0.1
1
10
100
1000
Time resolution (ns)
Single bunch in hybrid mode
Single bunch in 24-bunch mode
Super-bunch in hybrid mode
2 consecutive bunches in 24-bunch mode
3 consecutive bunches in 24-bunch mode
102 mA ring current

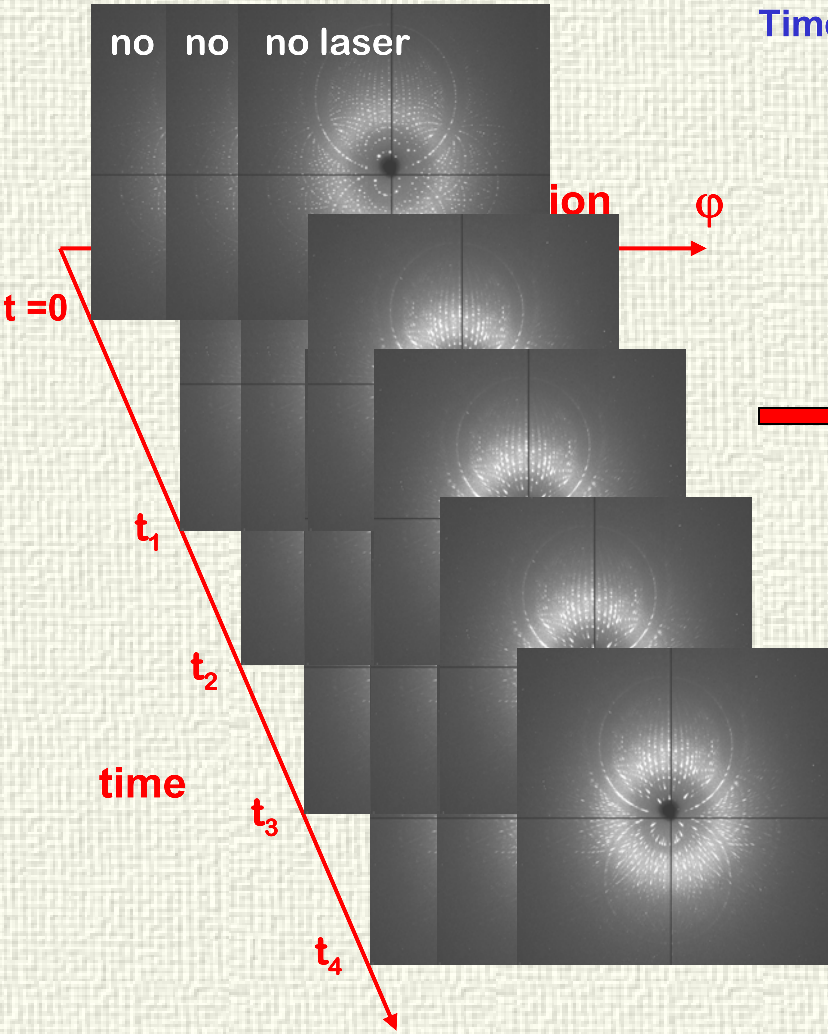






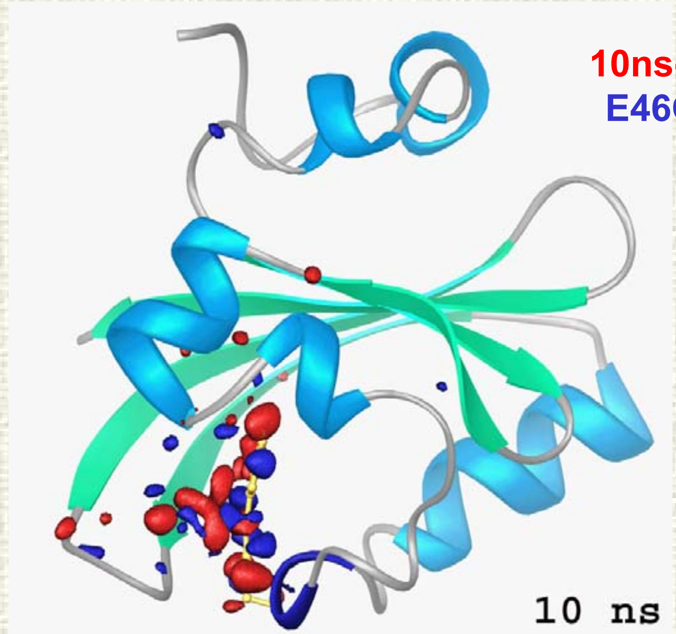


Friedrich Schotte, APS, 20 Sep 2007

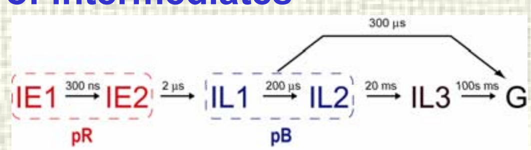


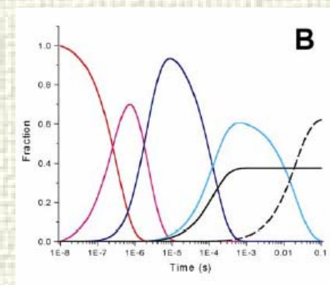
TR data collection

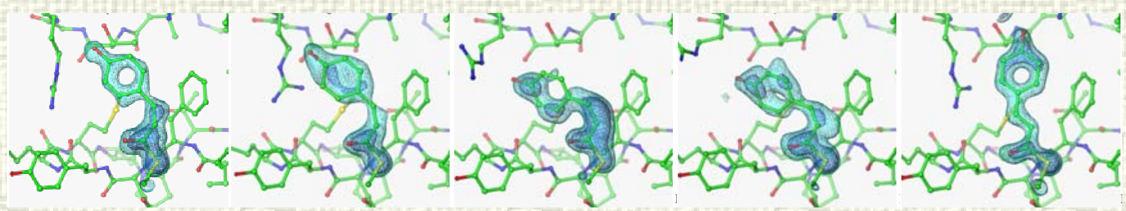
Time-dependent difference electron density map movie, Δρ (t)



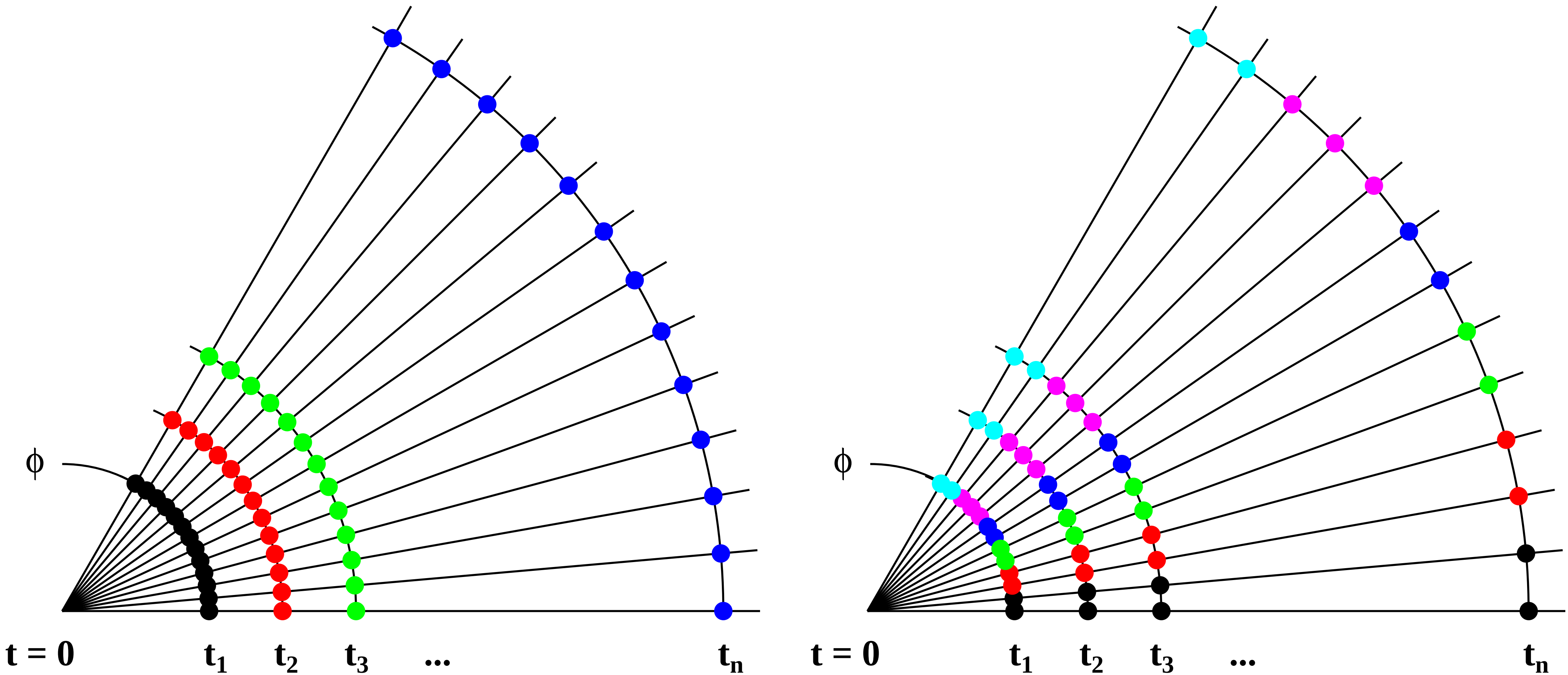
SVD/post-SVD analysis: mechanism & structures of intermediates



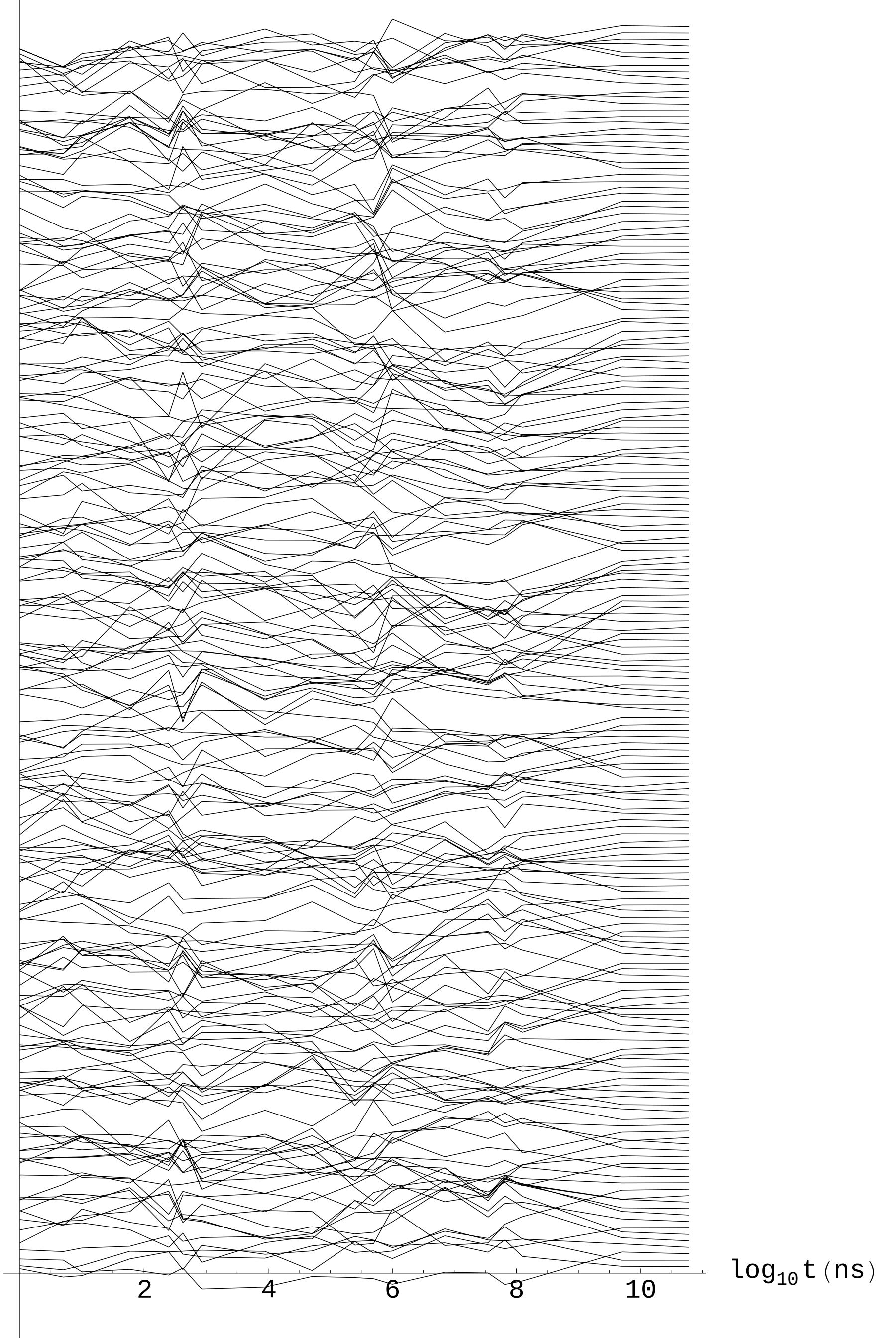




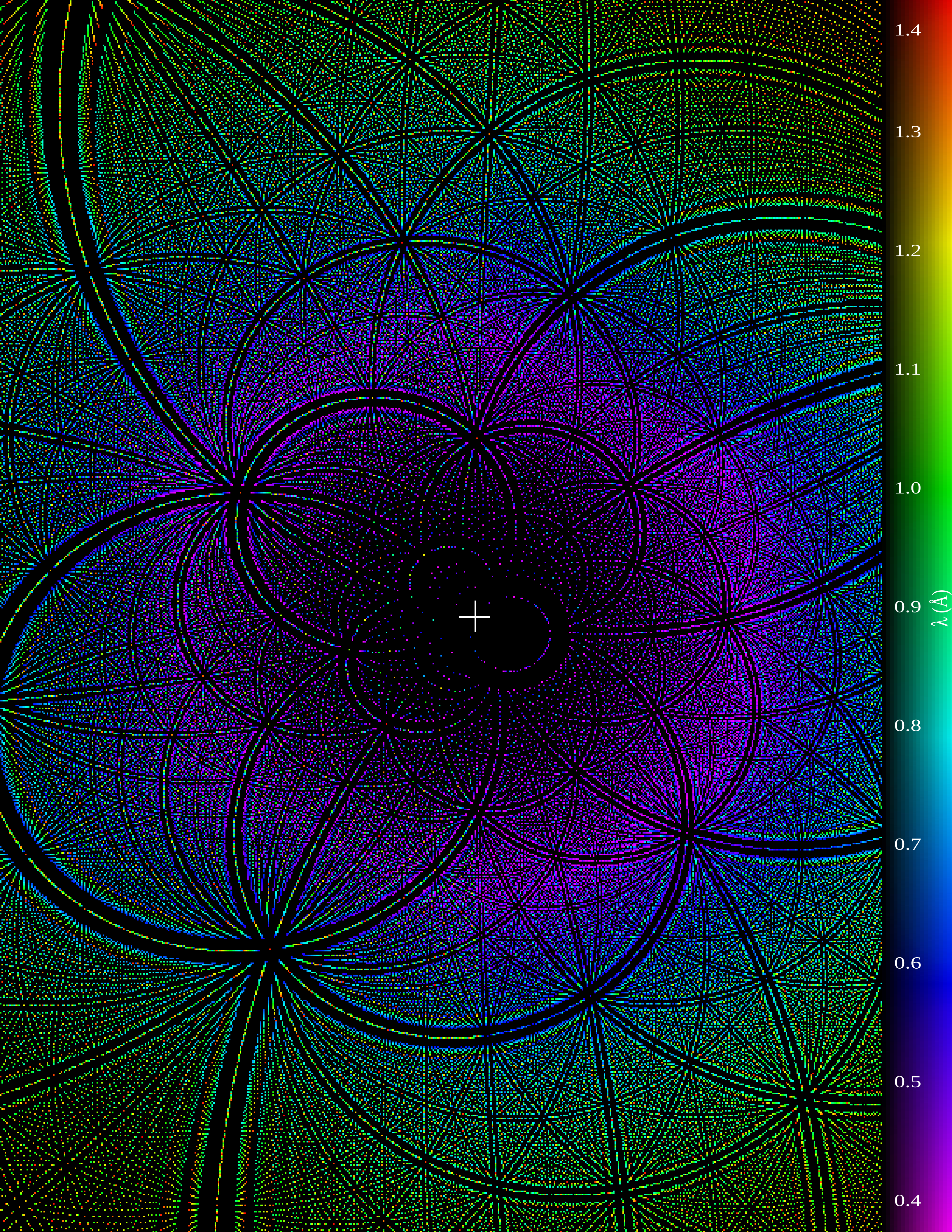
300ns 2μs 200-300μs 20ms >100ms t

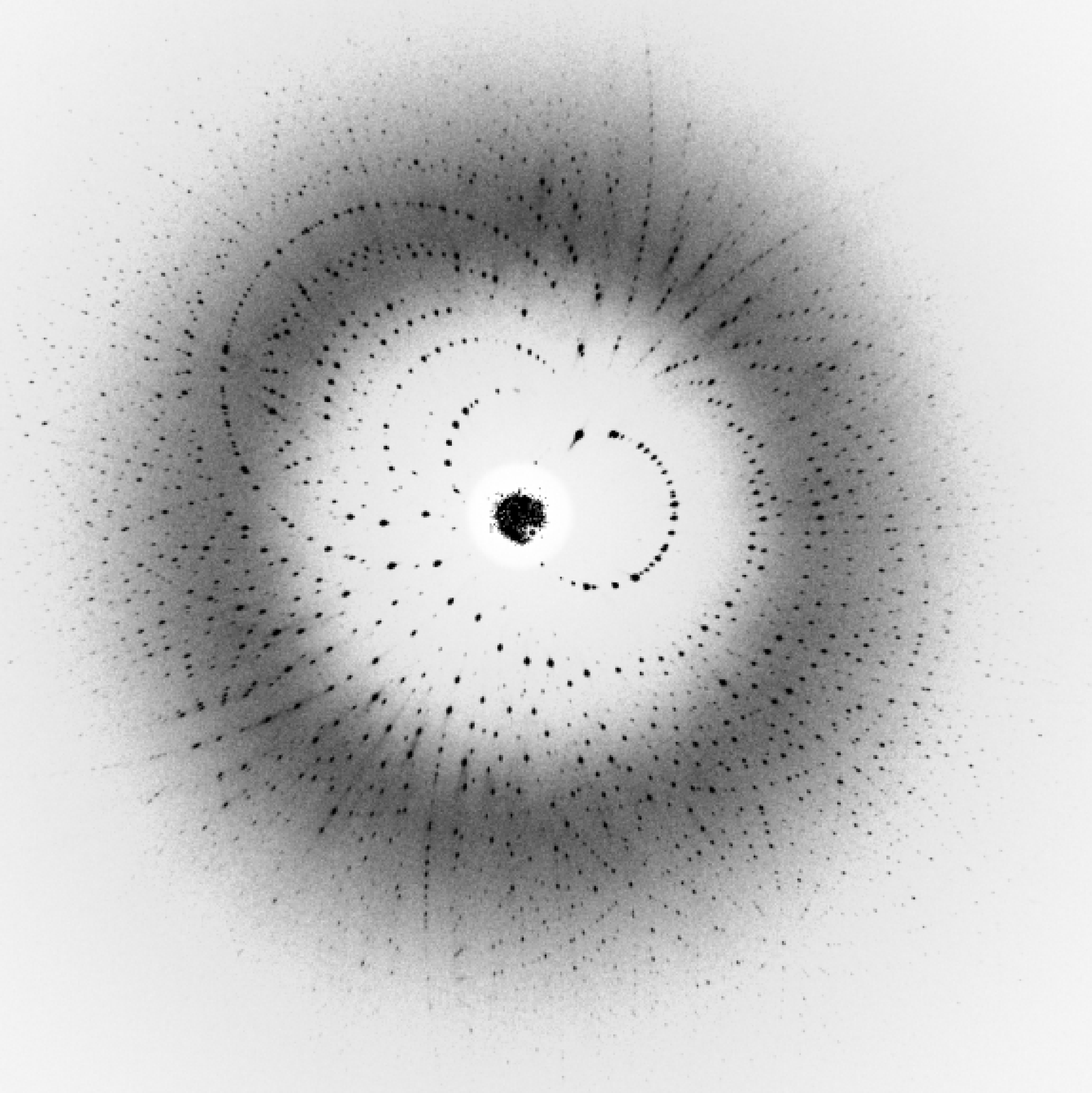


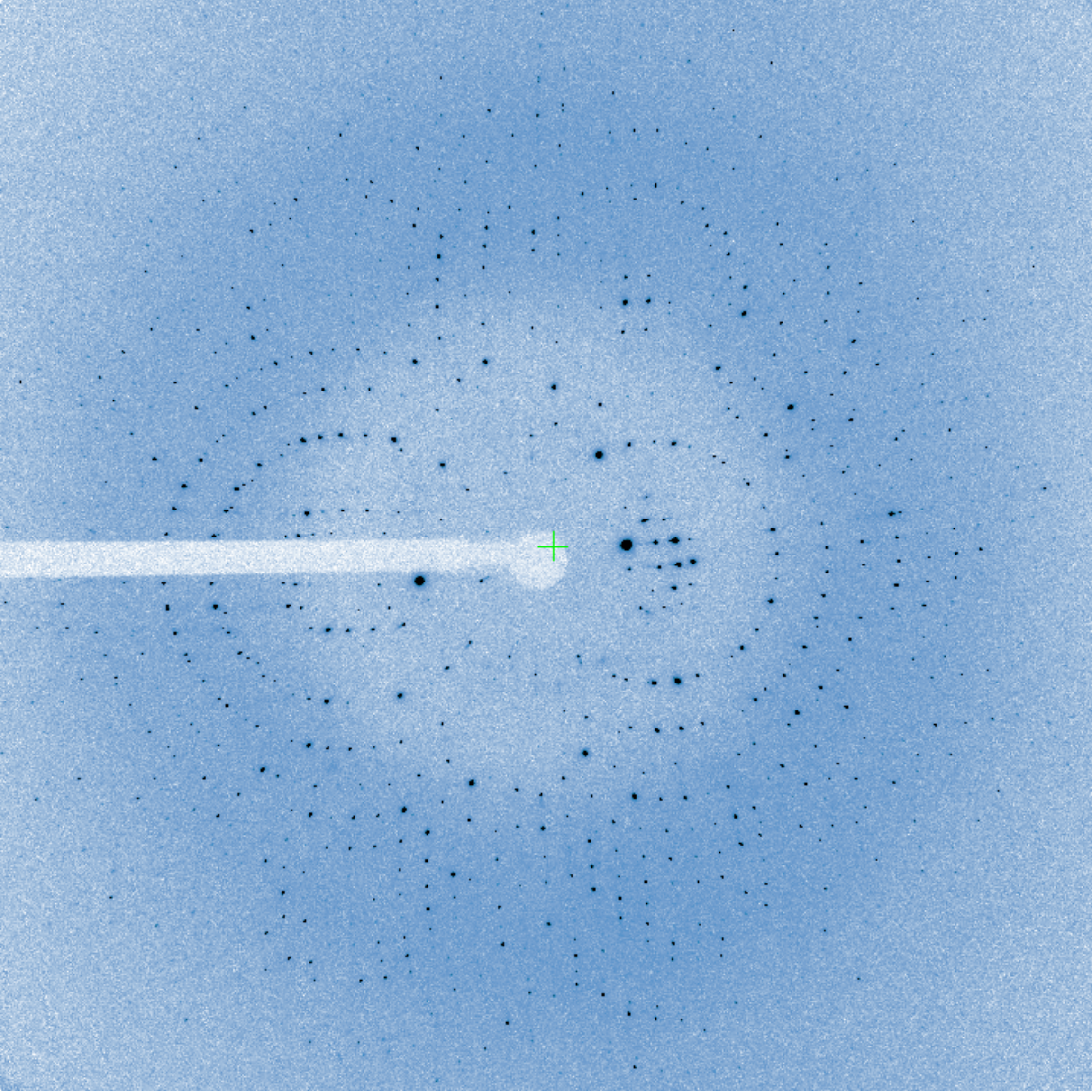
φ
t = 0
t1
t2
t3
...
tn
φ
t = 0
t1
t2
t3
...
tn

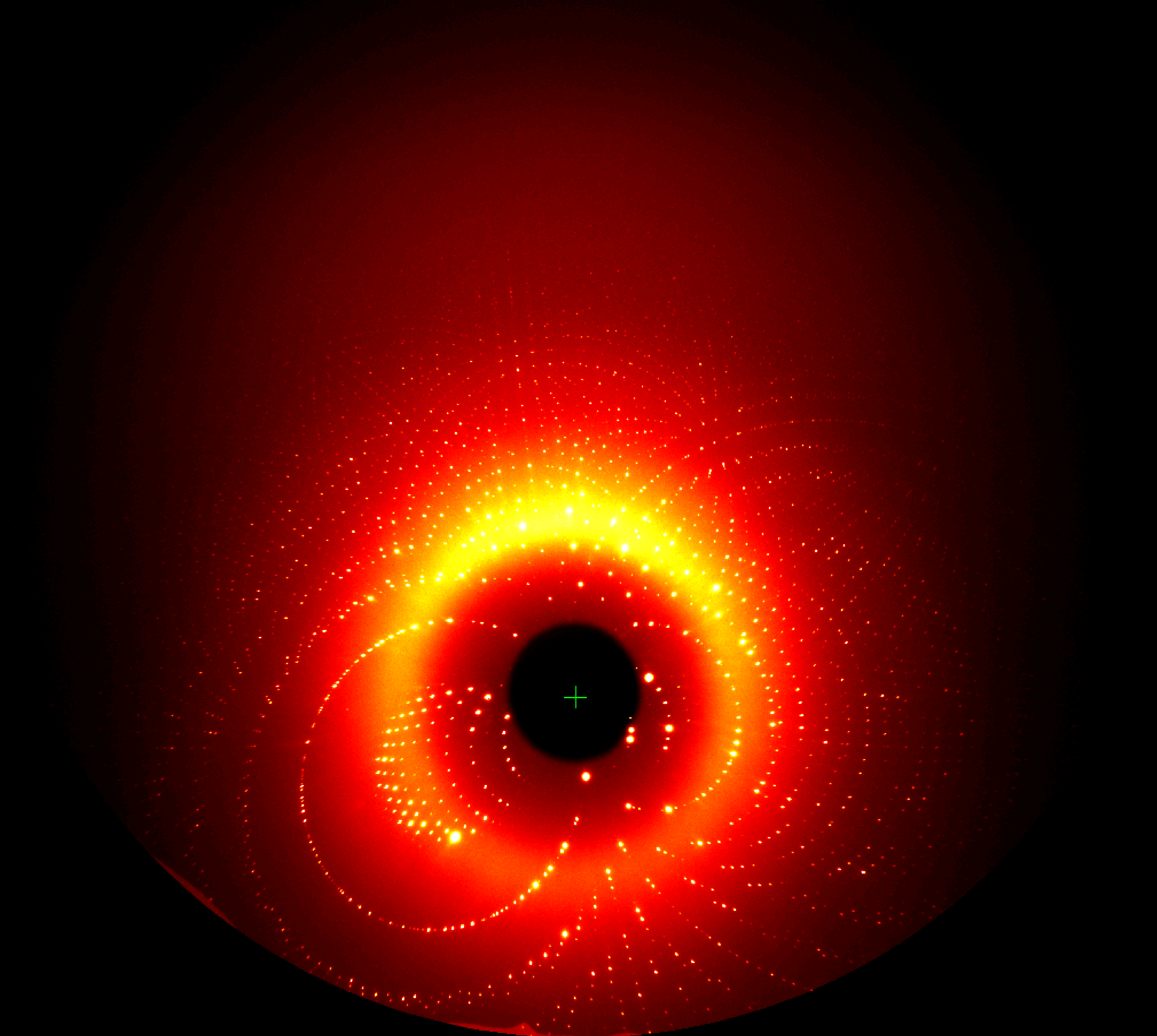


2
4
6
8
10
$\log_{10} t$ (ns)

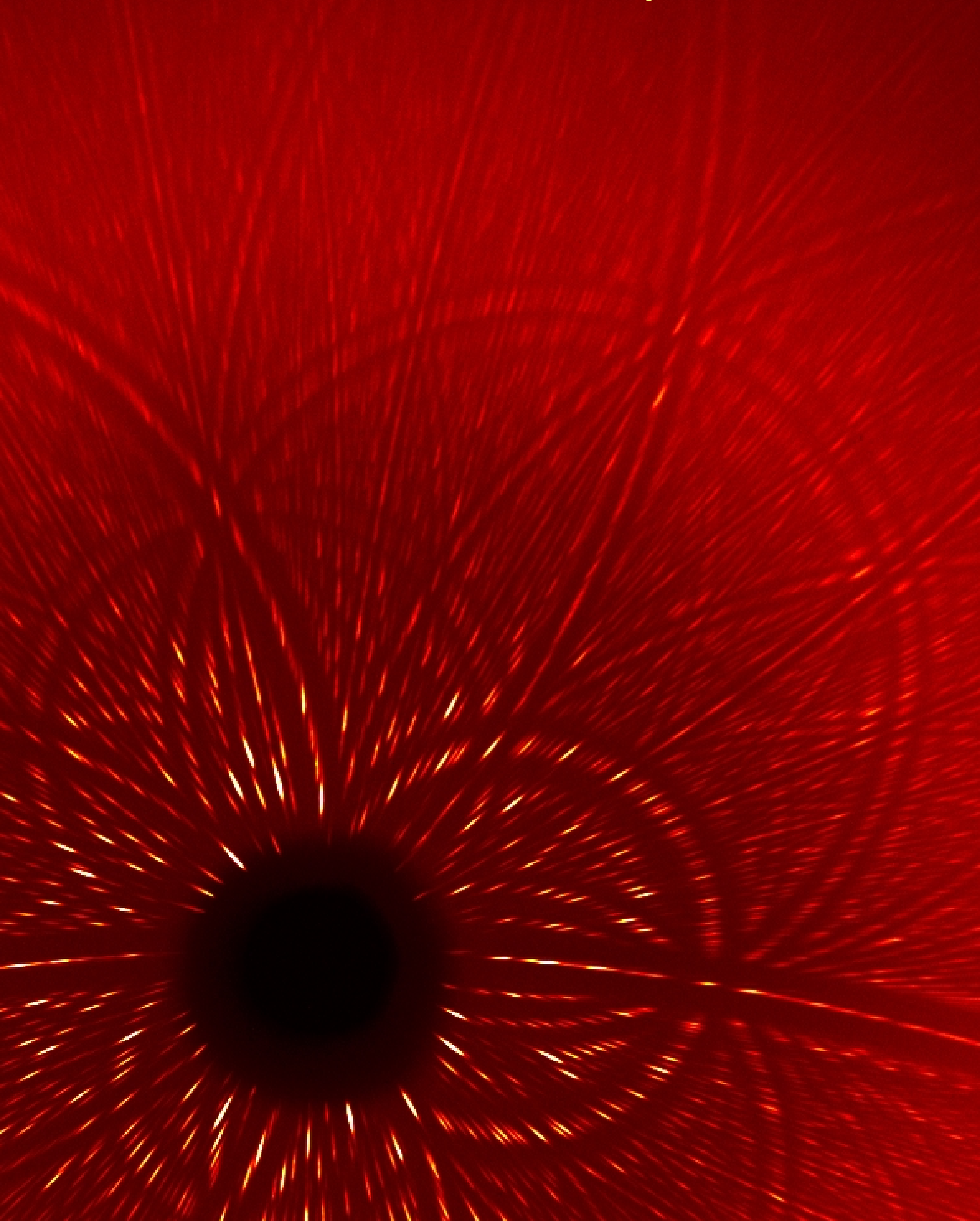

1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
λ (Å)

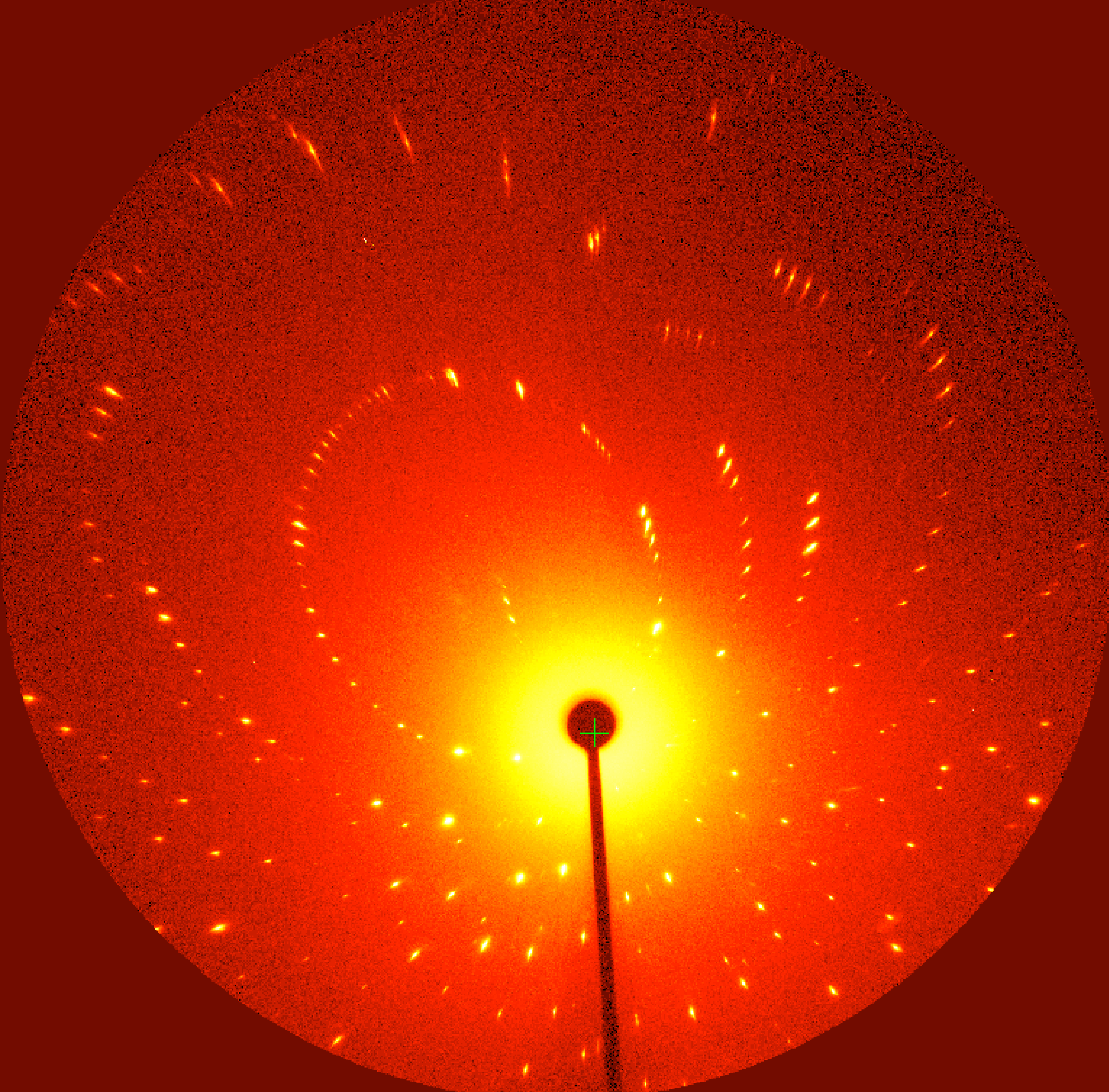


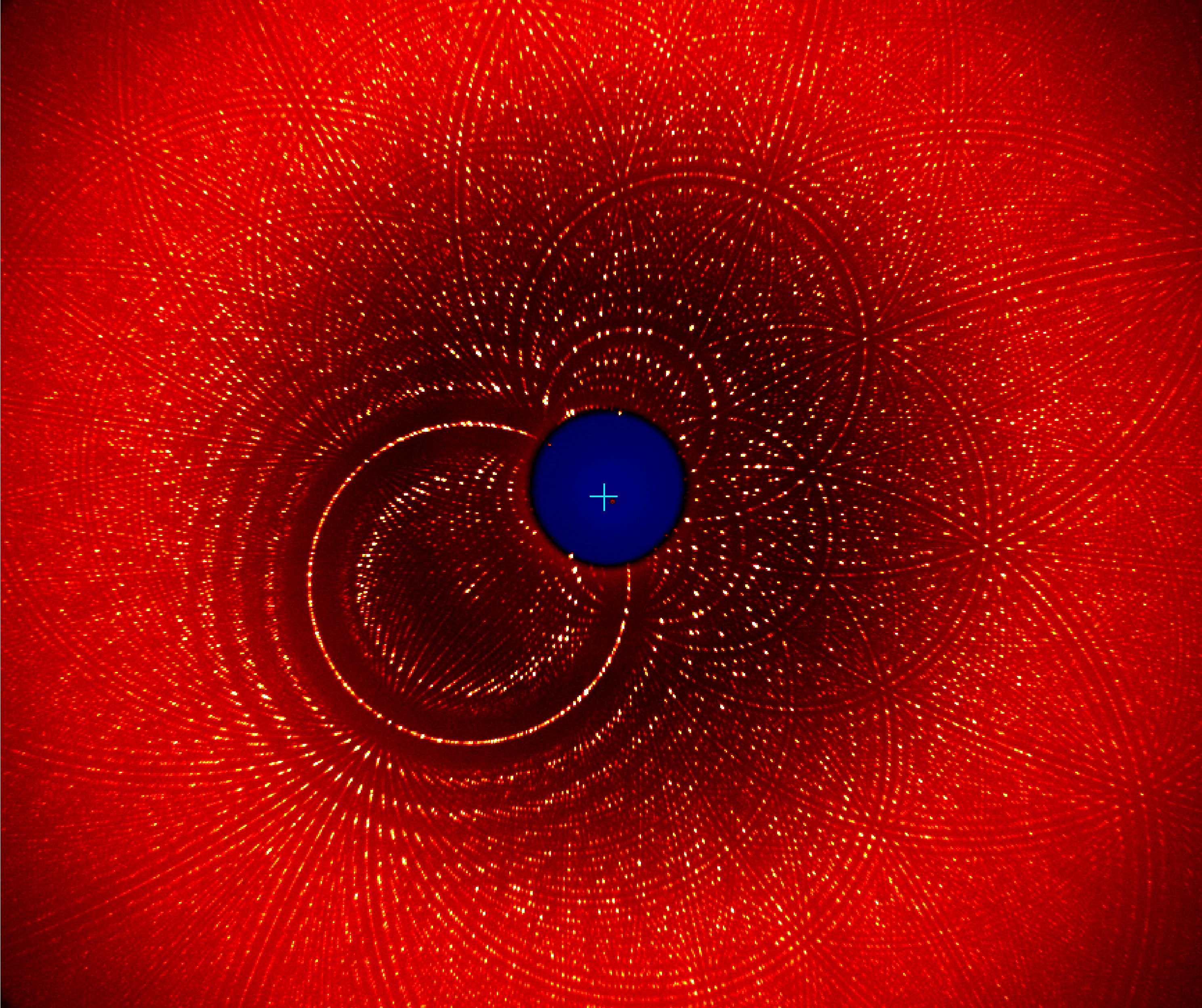


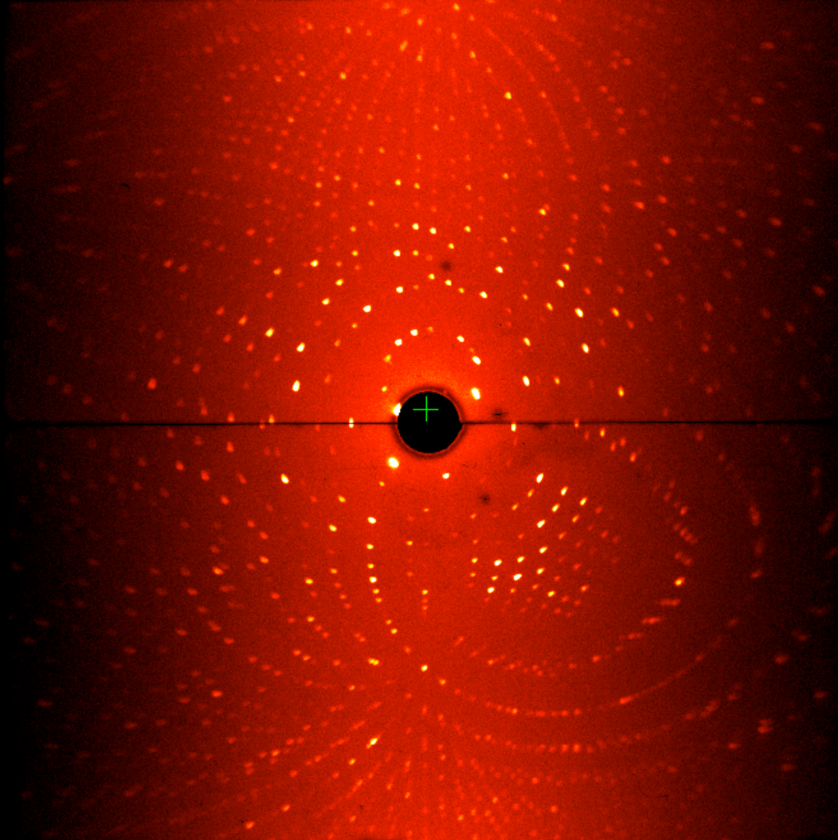


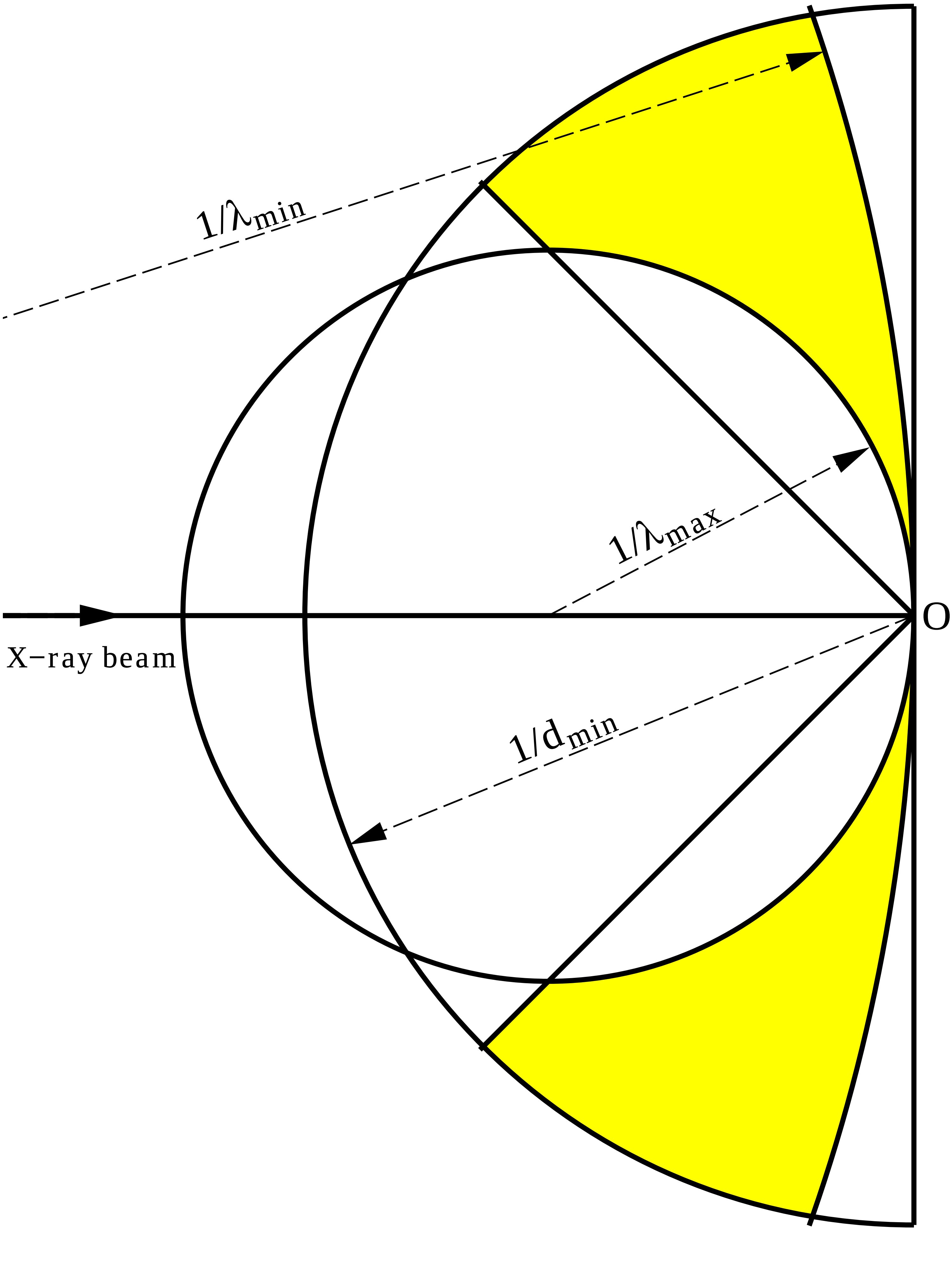
Laue diffraction from mosaic crystal





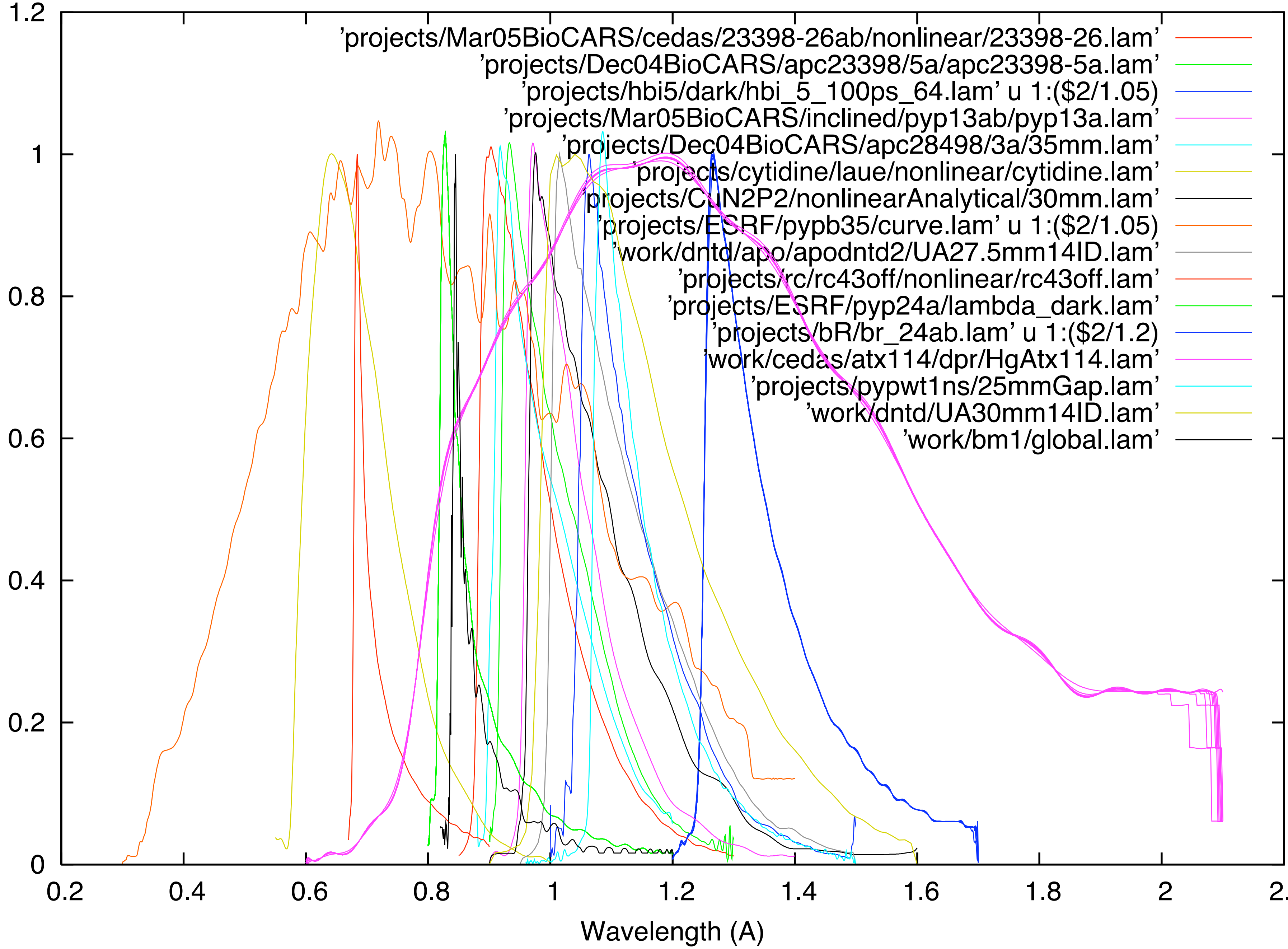






$1/\lambda_{min}$
$1/\lambda_{max}$
O
X−ray beam
$1/d_{min}$

animation



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'projects/Dec04BioCARS/apc23398/5a/apc23398-5a.lam'
'projects/hbi5/dark/hbi_5_100ps_64.lam' u 1:($2/1.05)
'projects/Mar05BioCARS/inclined/pyp13ab/pyp13a.lam'
'projects/Dec04BioCARS/apc28498/3a/35mm.lam'
'projects/cytidine/laue/nonlinear/cytidine.lam'
'projects/CuN2P2/nonlinearAnalytical/30mm.lam'
'projects/ESRF/pypb35/curve.lam' u 1:($2/1.05)
'work/dntd/apo/apodntd2/UA27.5mm14ID.lam'
'projects/rc/rc43off/nonlinear/rc43off.lam'
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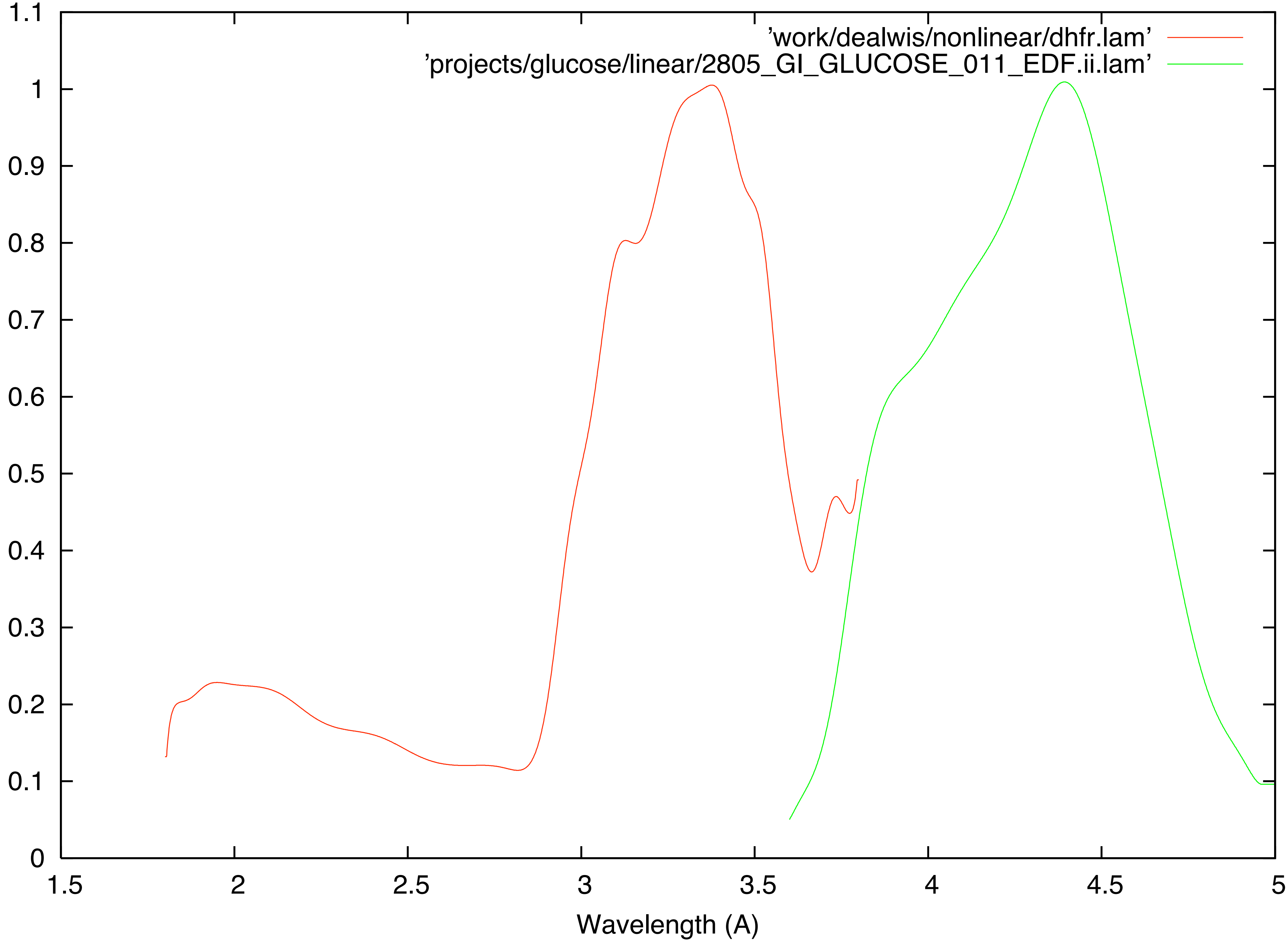
'projects/pypwt1ns/25mmGap.lam'
'work/dntd/UA30mm14ID.lam'
'work/bm1/global.lam'
1.2
1
0.8
0.6
0.4
0.2
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2
Wavelength (A)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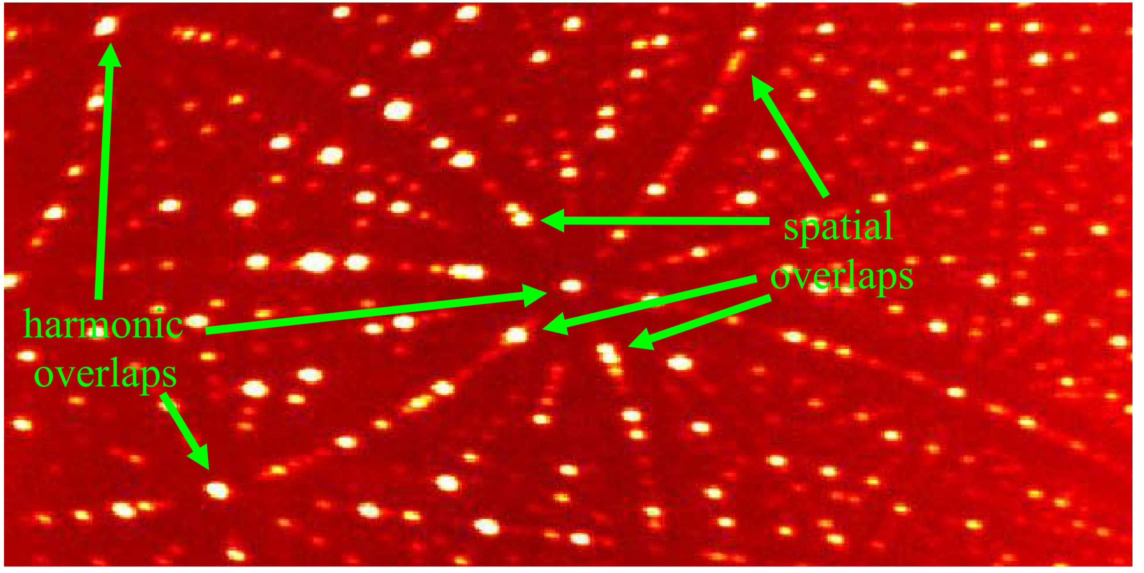
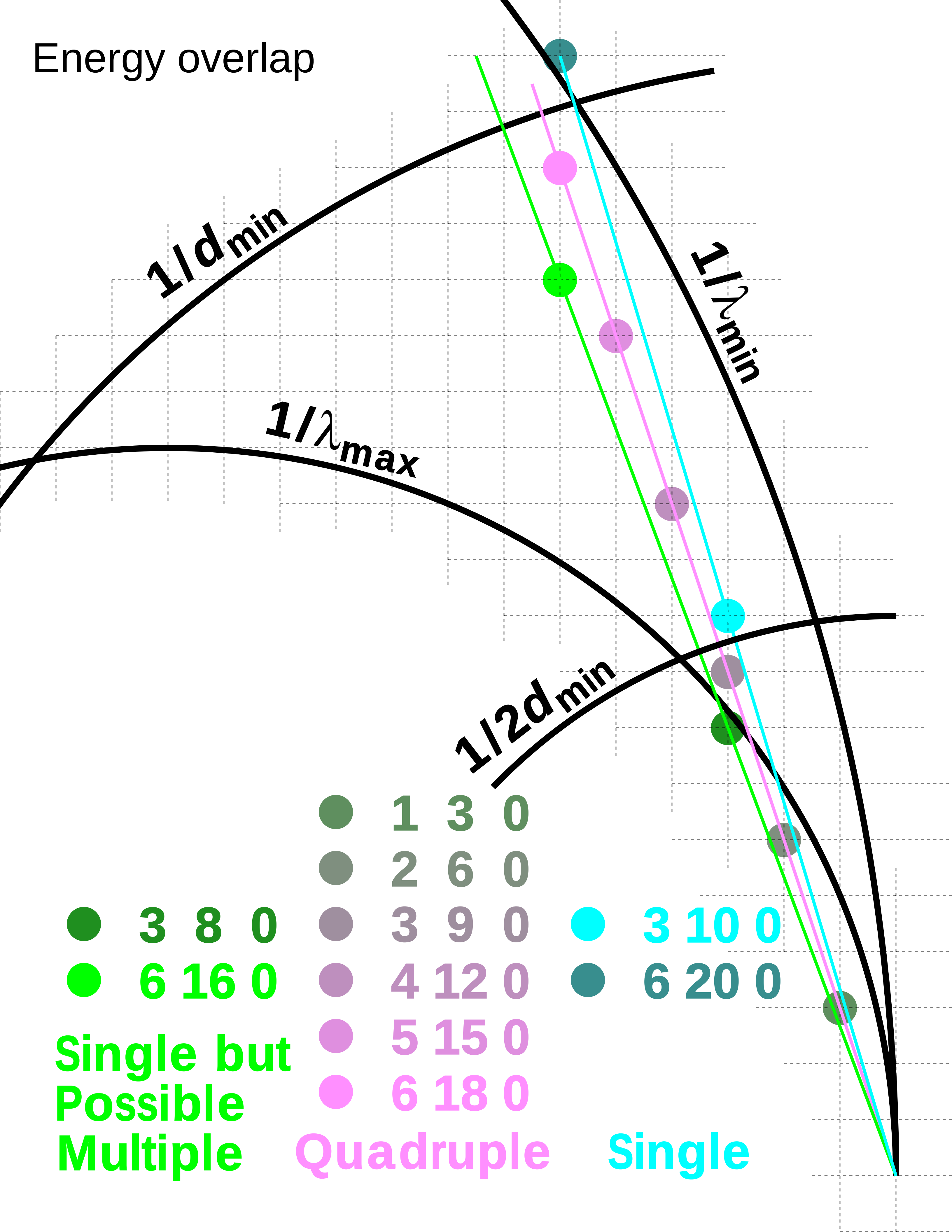
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1.5
2
2.5
3
3.5
4
4.5
5
Wavelength (A)

Harmonic and spatial overlap deconvolution

Harmonic reflections like (2 3 1) and (4 6 2) overlap exactly in one spot on a Laue pattern. A specially-designed numerical procedure called harmonic deconvolution is able to separate these two reflections. This process relies on redundant and symmetry-related measurements. The same algorithm is also applied to deconvolute extremely-close spatial overlaps. These procedures help to evaluate more data at both low and high resolution ranges. Data completeness can be improved by an additional 10-15%.




Energy overlap
1/dmin
1/λmin
1/λmax
1/2dmin
1 3 0
2 6 0
3 8 0
3 9 0
3 10 0
6 16 0
4 12 0
6 20 0
5 15 0
6 18 0
Single but Possible Multiple
Quadruple
Single

Laue software

- Daresbury Laboratory Laue Software Suite (http://www.srs.ac.uk/px/jwc_laue/laue_top.html)
- TReX, Friedrich Schotte
- LEAP (Laue Evaluation Analysis Package), Soichi Wakatsuki
- LAUECELL, Raimond B.G. Ravelli (http://www.crystal.chem.uu.nl/distr/man_lauecell/lauecell.html)
- LaueView, Zhong Ren (http://cars.uchicago.edu/biocars/pages/lauemanuals.shtml)
- Precognition™, Zhong Ren, Renz Research, Inc. (http://renzresearch.com/Precognition)

Key points in Laue data processing

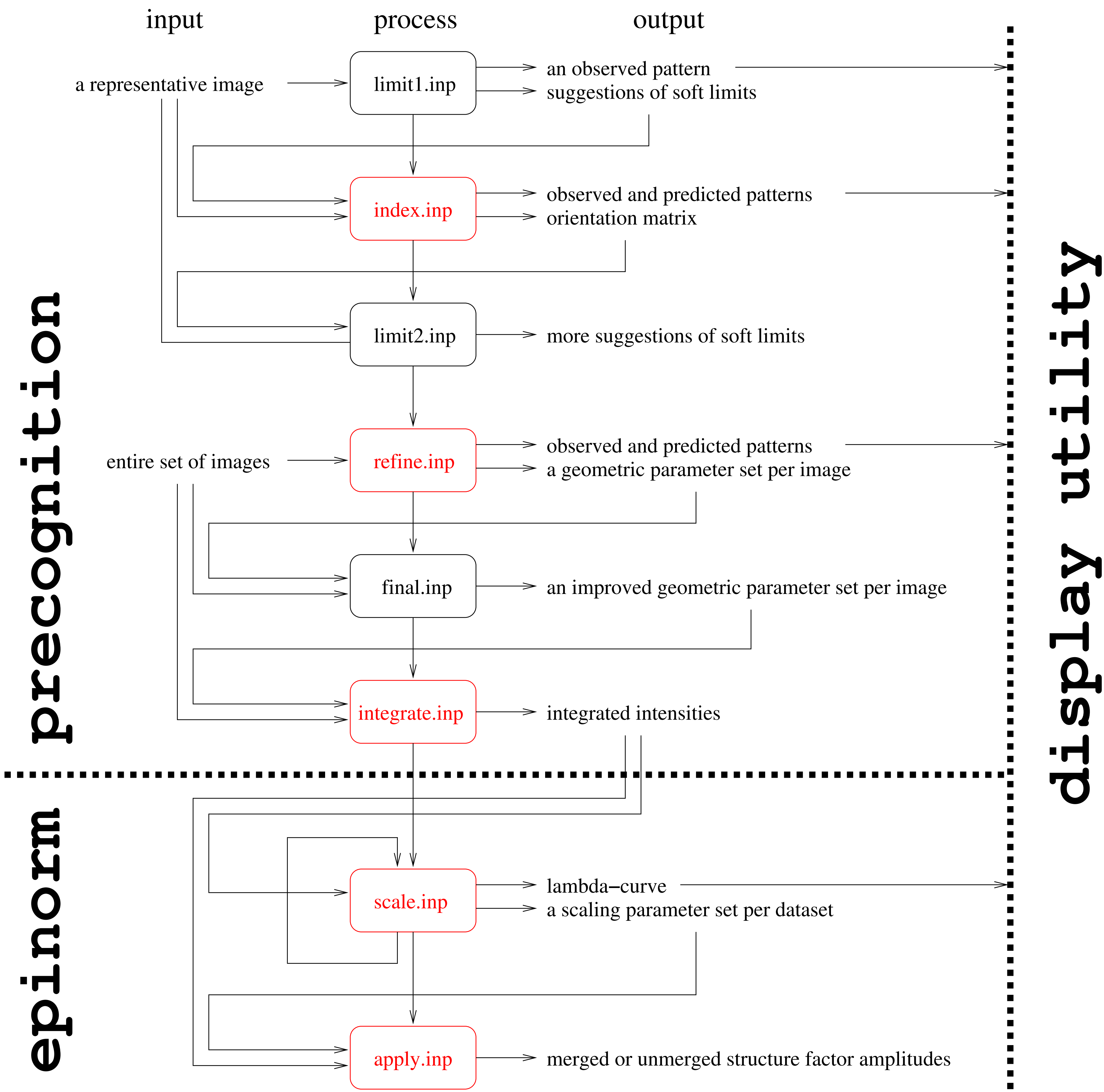
Geometry: Precise prediction of the location of each and every diffraction spot is critical to all subsequent processing.

Integration: Faithful integration of recorded pixel intensities and accurate evaluation of local background are keys to capture of structural signal.

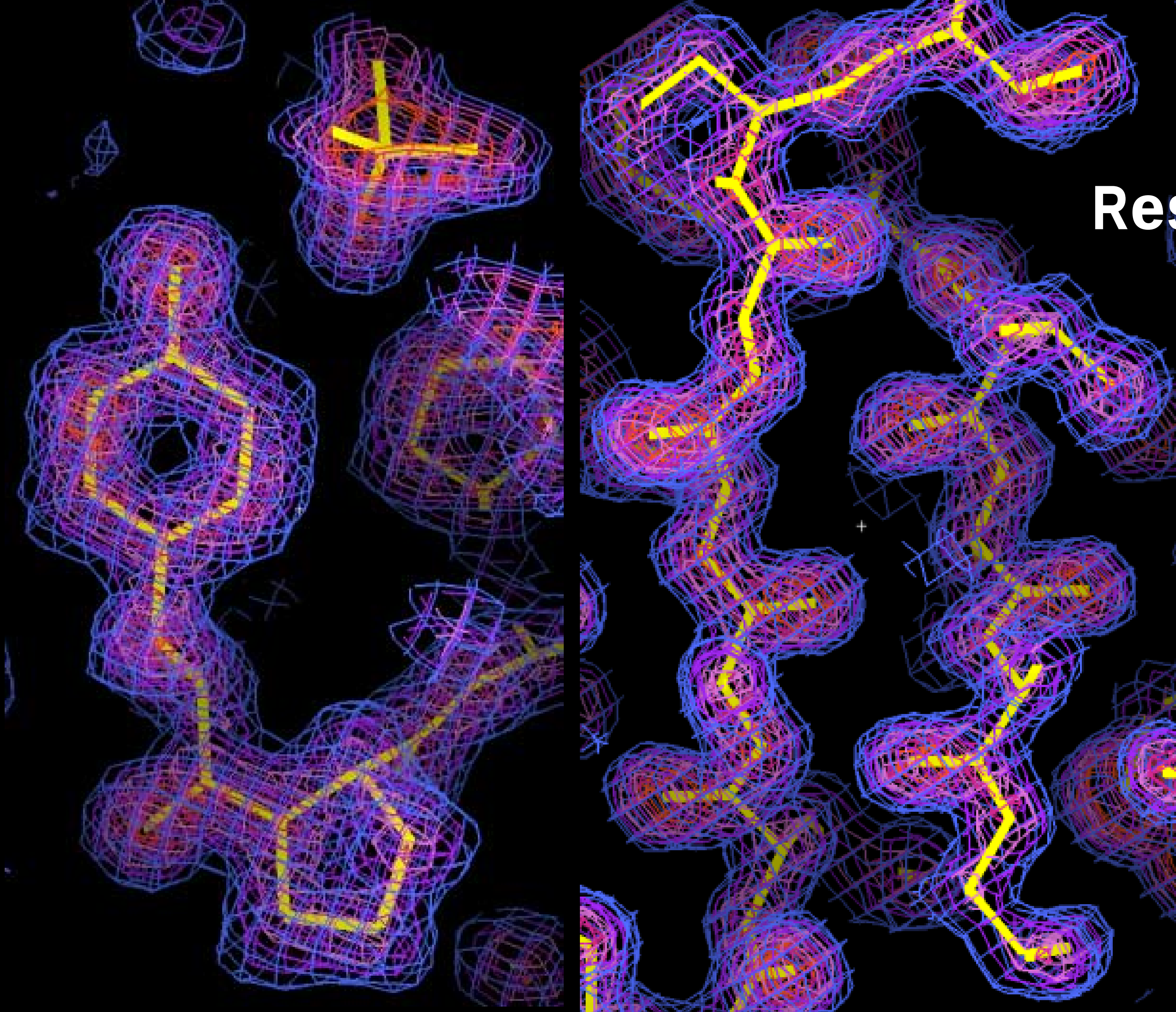
Scaling: Ability to model a variety of systematic errors during the complex process of wavelength normalization and scaling leads to precise data reduction.

Mosaicity: Proper handling of crystal mosaicity ensures smooth processing in various steps.

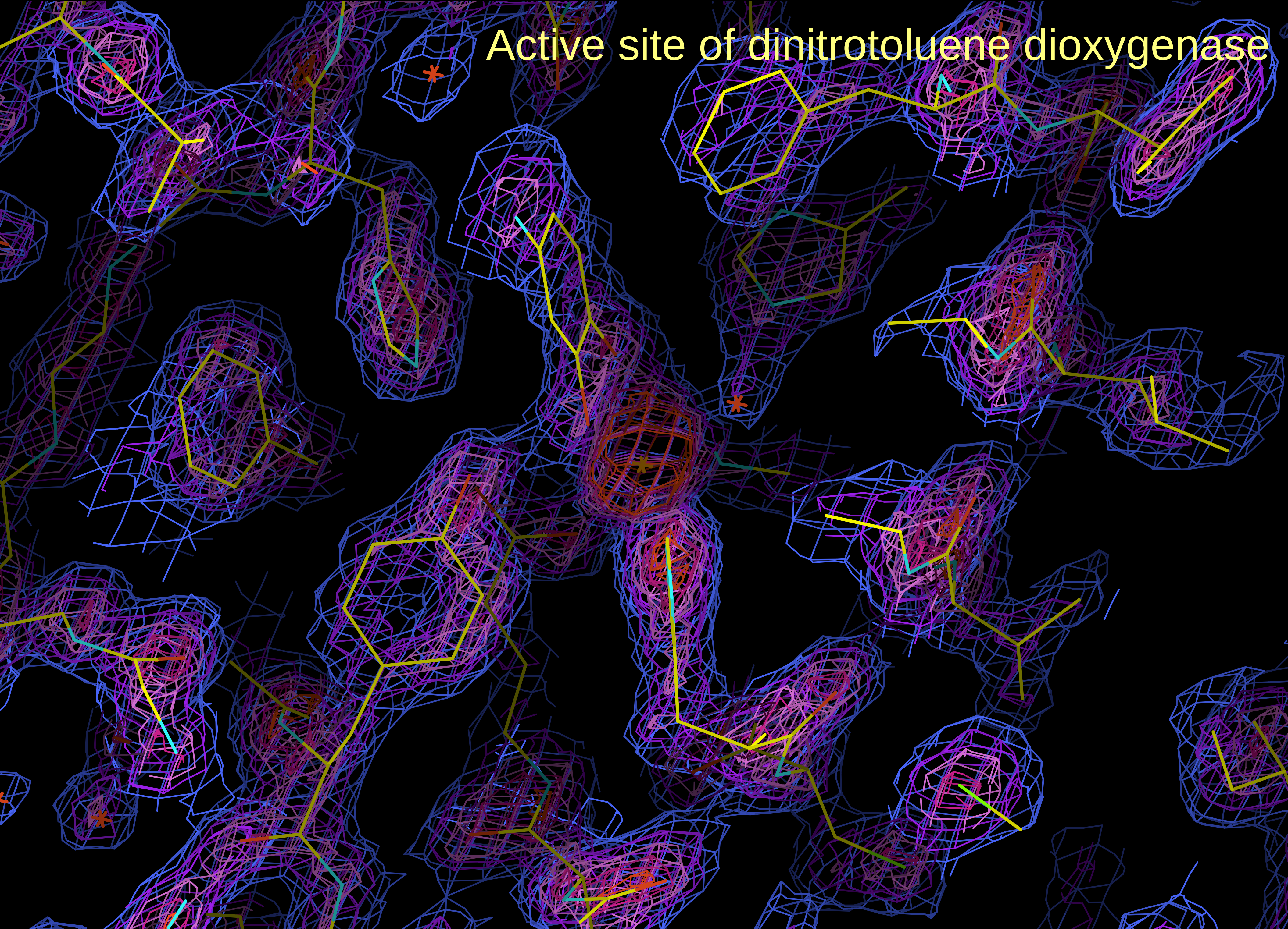
Overlap: Sophisticated deconvolution procedures can help to retrieve useful data otherwise lost in overlapping, and to achieve the best possible data completeness.



input
process
output
a representative image
limit1.inp
an observed pattern
suggestions of soft limits
index.inp
observed and predicted patterns
orientation matrix
limit2.inp
more suggestions of soft limits
entire set of images
refine.inp
observed and predicted patterns
a geometric parameter set per image
final.inp
an improved geometric parameter set per image
integrate.inp
integrated intensities
scale.inp
lambda−curve
a scaling parameter set per dataset
apply.inp
merged or unmerged structure factor amplitudes
precognition
epinorm
display utility

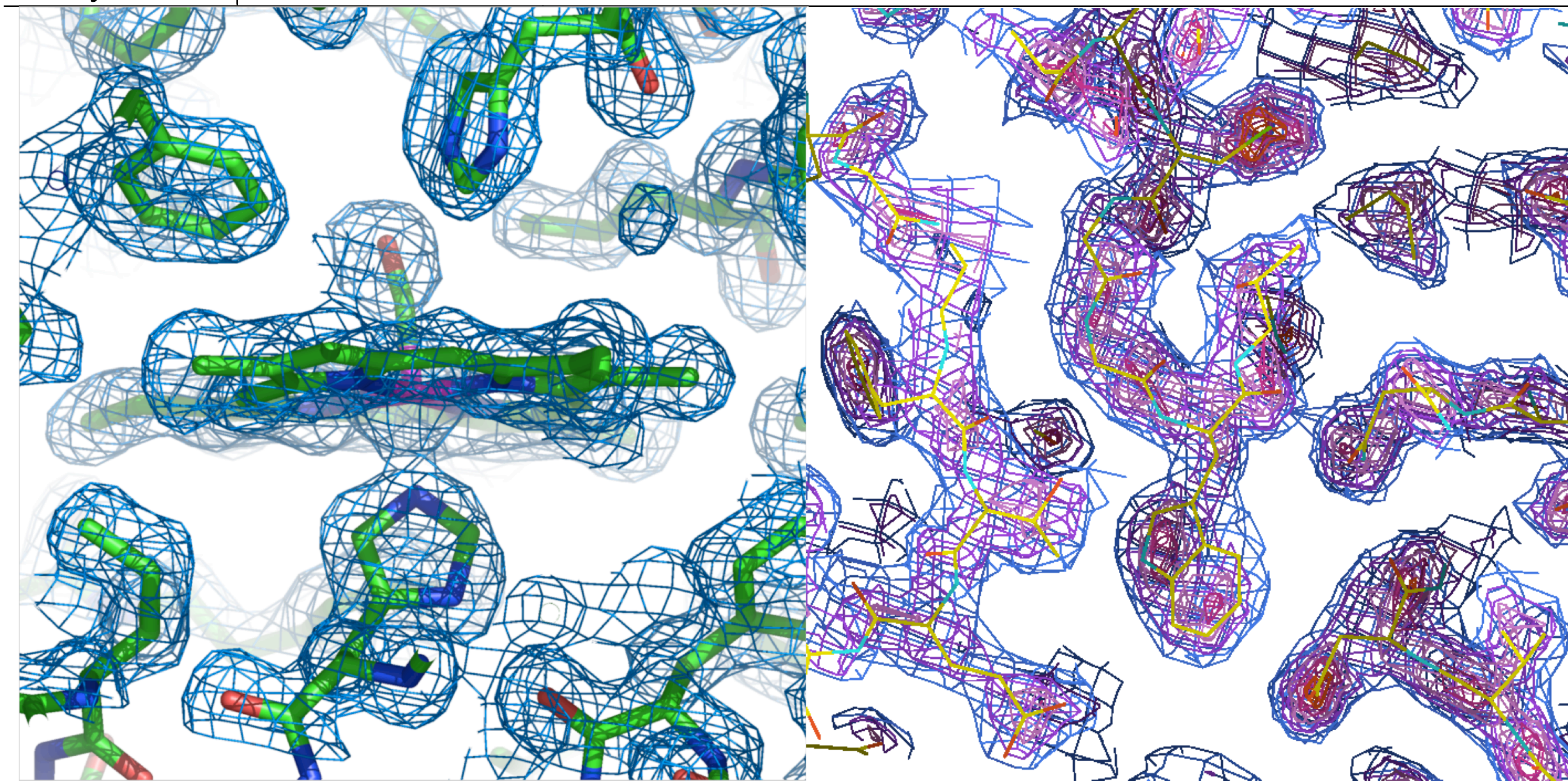


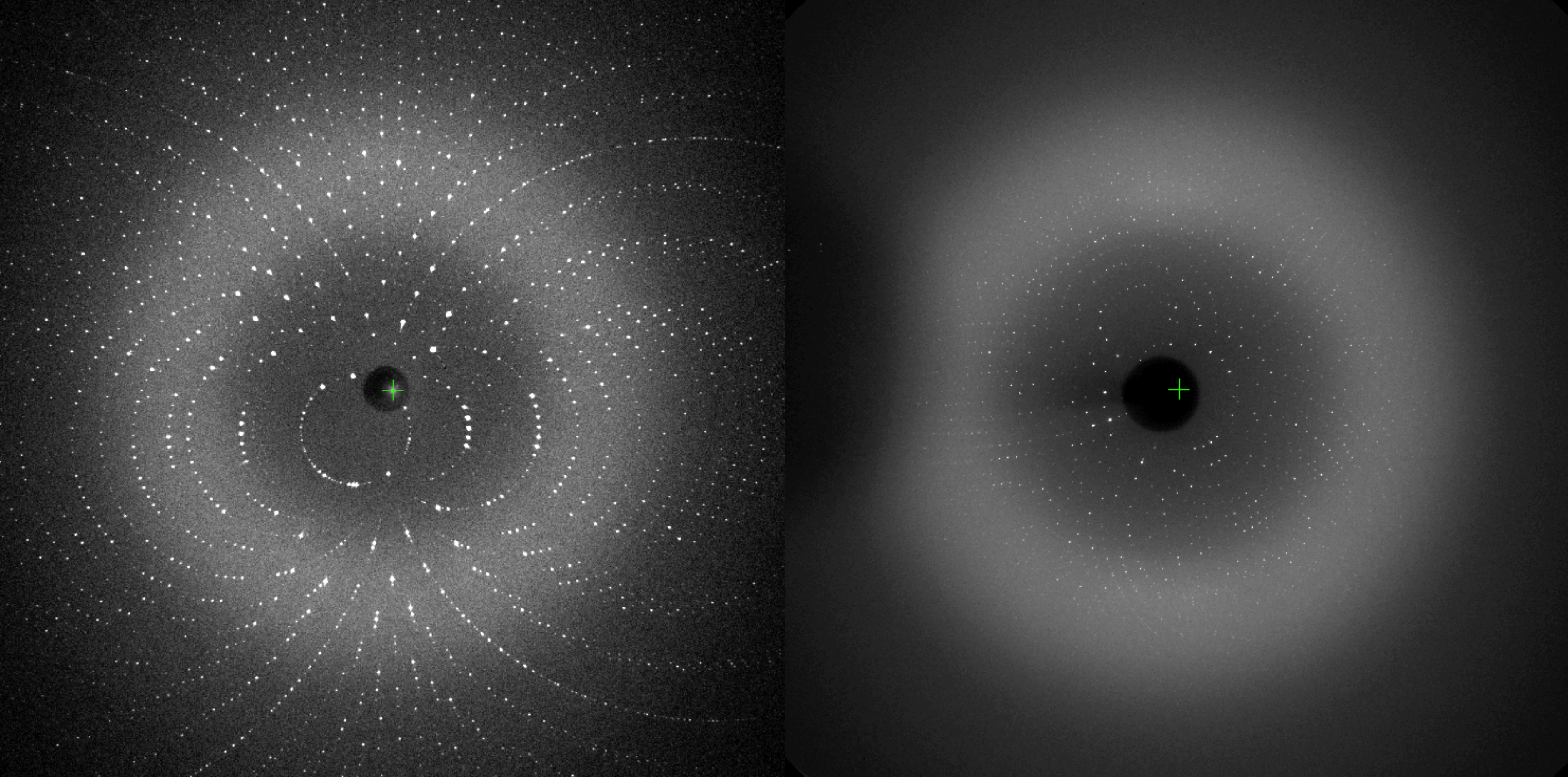
Restrictocin
$2F_o - F_c$
maps
at
1.7 A

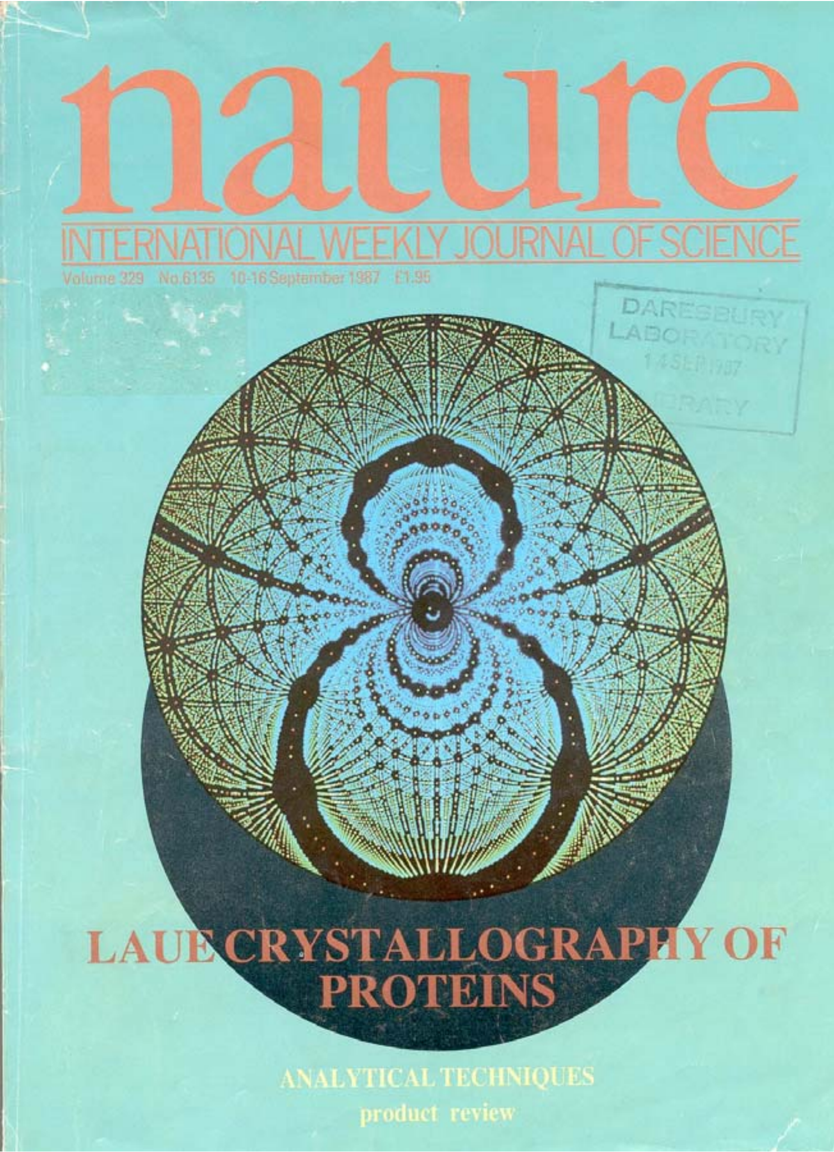

Active site of dinitrotoluene dioxygenase

Structure refinement against Laue data

Structure	Images	Resolution	Completeness	R_{cryst}	R_{free}	Bond length rmsd	Bond angle rmsd
Hemoglobin	62	1.6 Å	85.9%	14.7%	16.7%	0.010 Å	0.068°
Exohydrolase	32	2.6 Å	75.9%	19.5%	25.8%	0.025 Å	2.15°







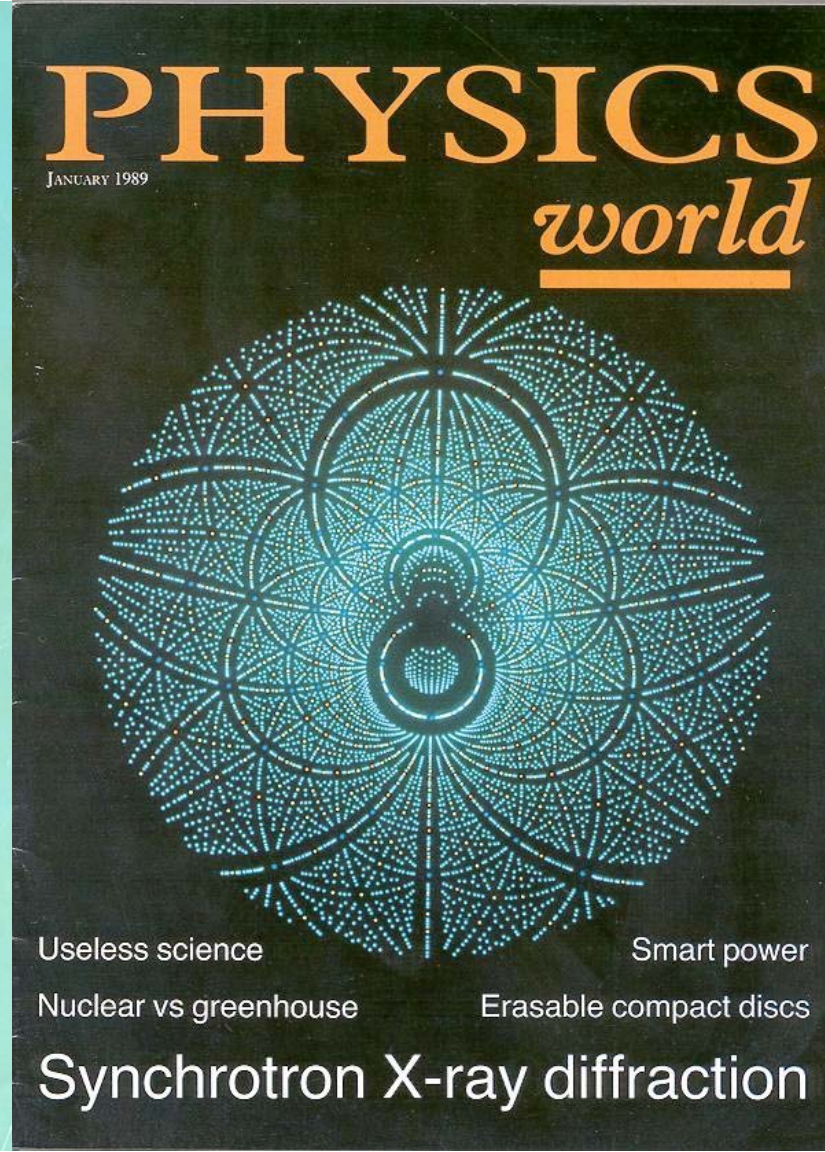
nature

INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

Volume 329 No.6135 10-16 September 1987 £1.95

LAUE CRYSTALLOGRAPHY OF PROTEINS

ANALYTICAL TECHNIQUES
product review



PHYSICS world

JANUARY 1989

Useless science

Smart power

Nuclear vs greenhouse

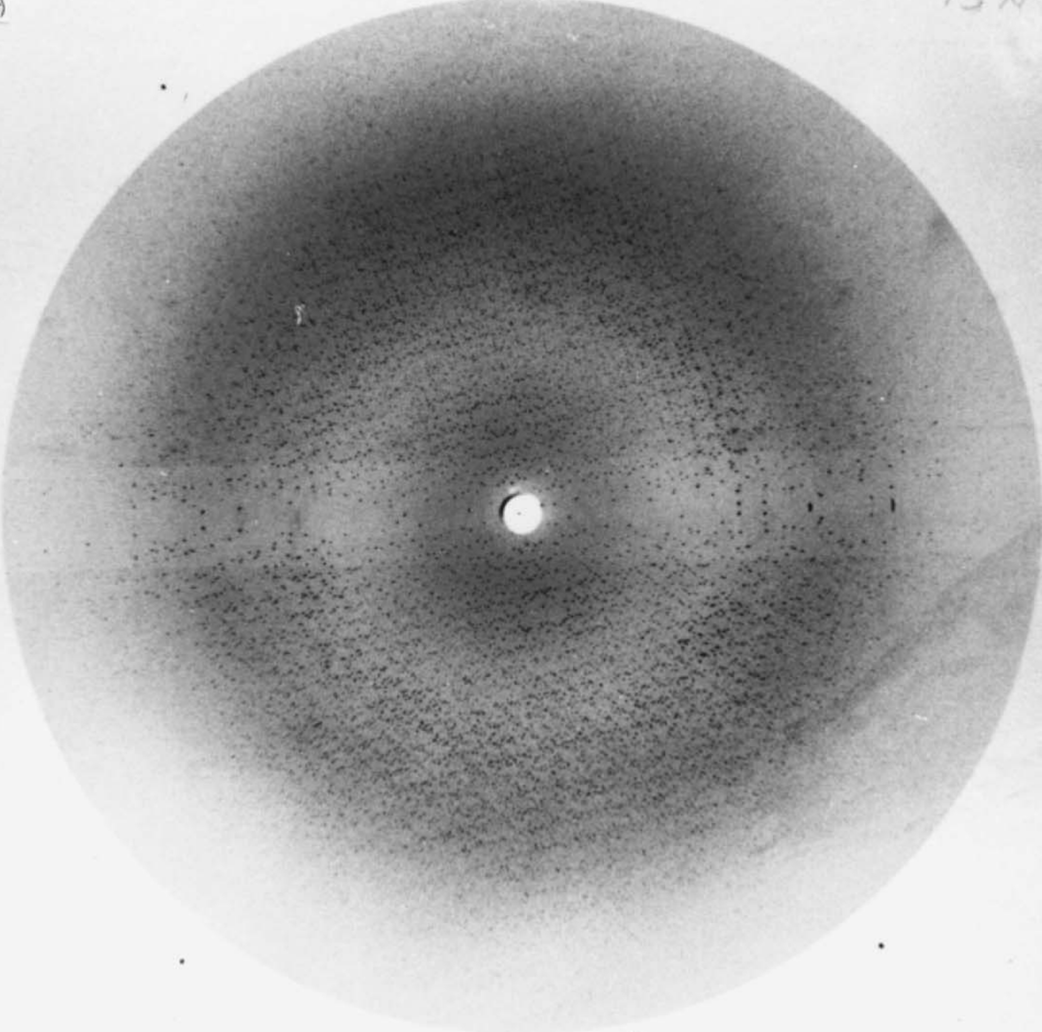
Erasable compact discs

Synchrotron X-ray diffraction

J. CHEM. SOC. FARADAY TRANS., 1993, 89(15), 2667–2675

High-resolution Crystallographic Studies of Native Concanavalin A using Rapid Laue Data Collection Methods and the Introduction of a Monochromatic Large-angle Oscillation Technique (LOT)

Susanne Weisgerber
Department of Chemistry, University of Manchester, UK M13 9PL
John R. Helliwell
*SERC, Daresbury Laboratory, Warrington, UK WA4 4AD
and Department of Chemistry, University of Manchester, UK M13 9PL*



J. Synchrotron Rad. (1997). **4**, 14–16

Time-Resolved Protein Crystallography with Large-Angle Oscillations: an Application of a Protein Data-Collection System Using the Weissenberg Technique and a Large-Format Imaging Plate

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(*Received 12 July 1996; accepted 6 November 1996*)

A diffraction-intensity data-collection system with synchrotron radiation X-rays utilizing the screenless Weissenberg technique and incorporating a large-format imaging plate is one of the most suitable apparatus for time-resolved protein crystallography with larger angle oscillations than hitherto described. The time resolution and data quality of the system have been tested using a tetragonal lysozyme crystal as a test sample in a flow-cell experiment at the bending-magnet beamline 18B at the Photon Factory, and a time resolution of 15 min is confirmed.

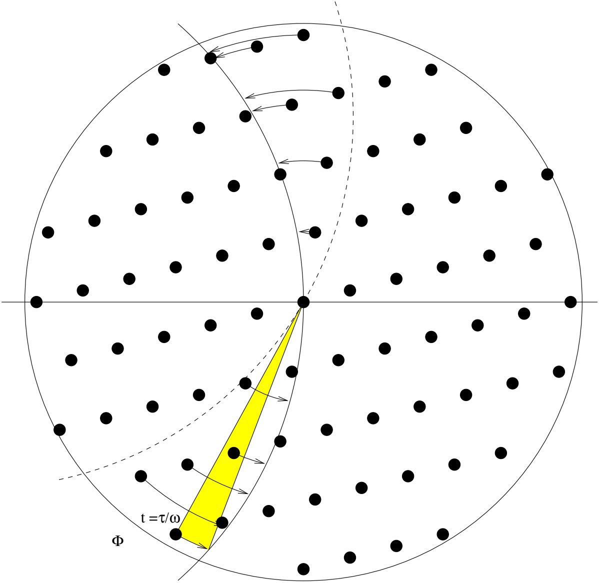
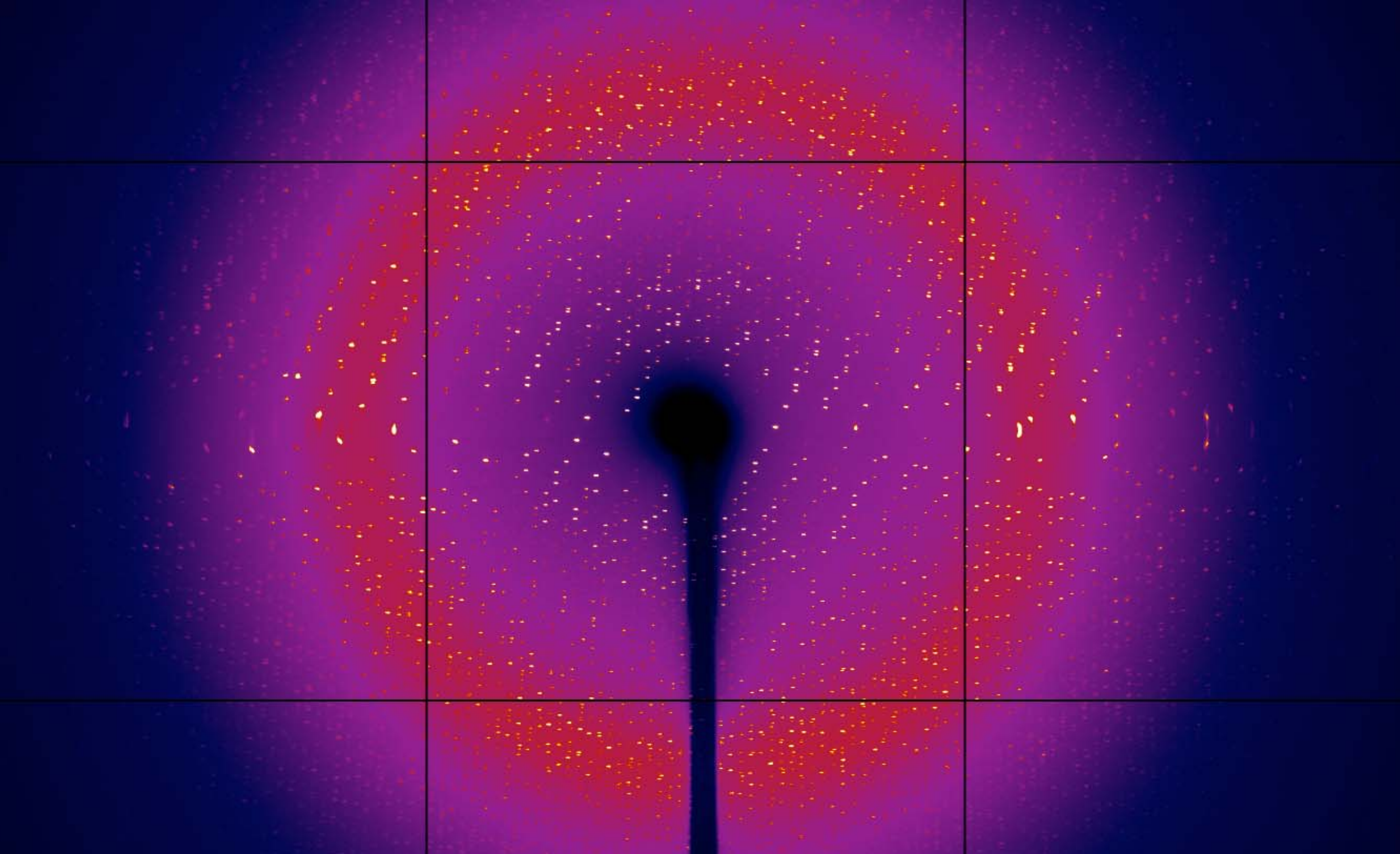


Figure 2.3.2.2.2.a A schematic drawing of reciprocal space in rotation geometry.

animation



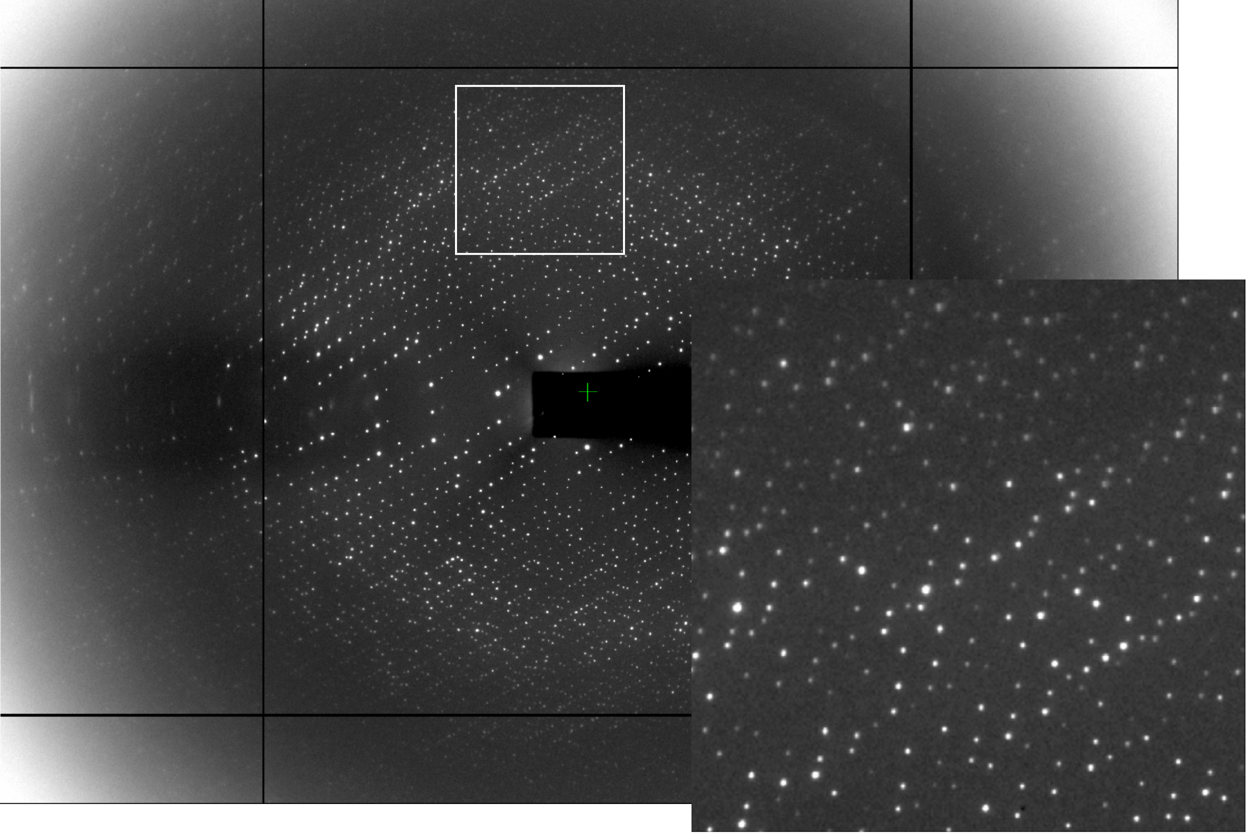


Figure 2.3.2.2.2.b A 30° LARG image from a bacteriophytochrome crystal showing adequate spot separation. The crystal was rotated about a horizontal axis. The inset is a blow-up of the boxed region in the main image, and illustrates the excellent spot separation.

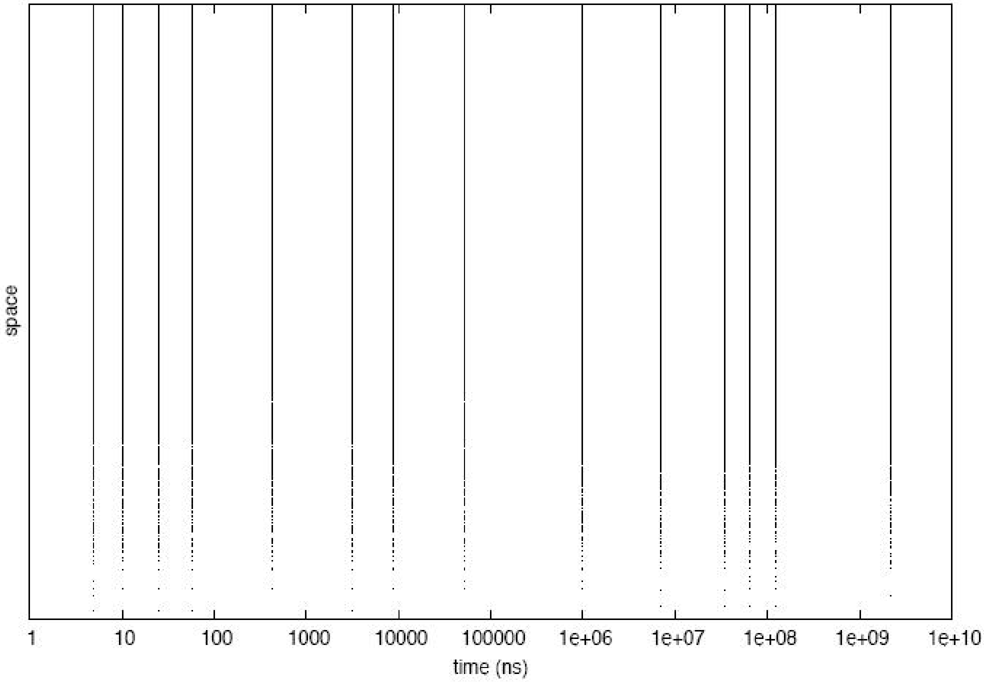


Figure 2.3.2.2.2.c Four-dimensional data distribution of a conventional, pump – probe Laue experiment. Three-dimensional reciprocal space is collapsed into a single dimension (the vertical axis) and the 4th dimension of time is shown as log t (the horizontal axis).

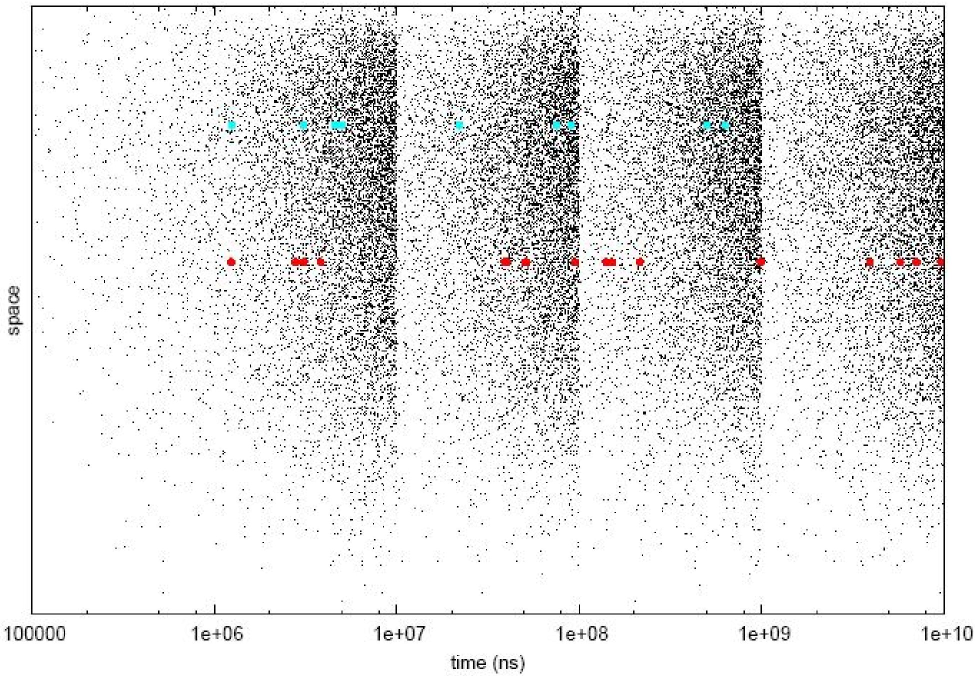
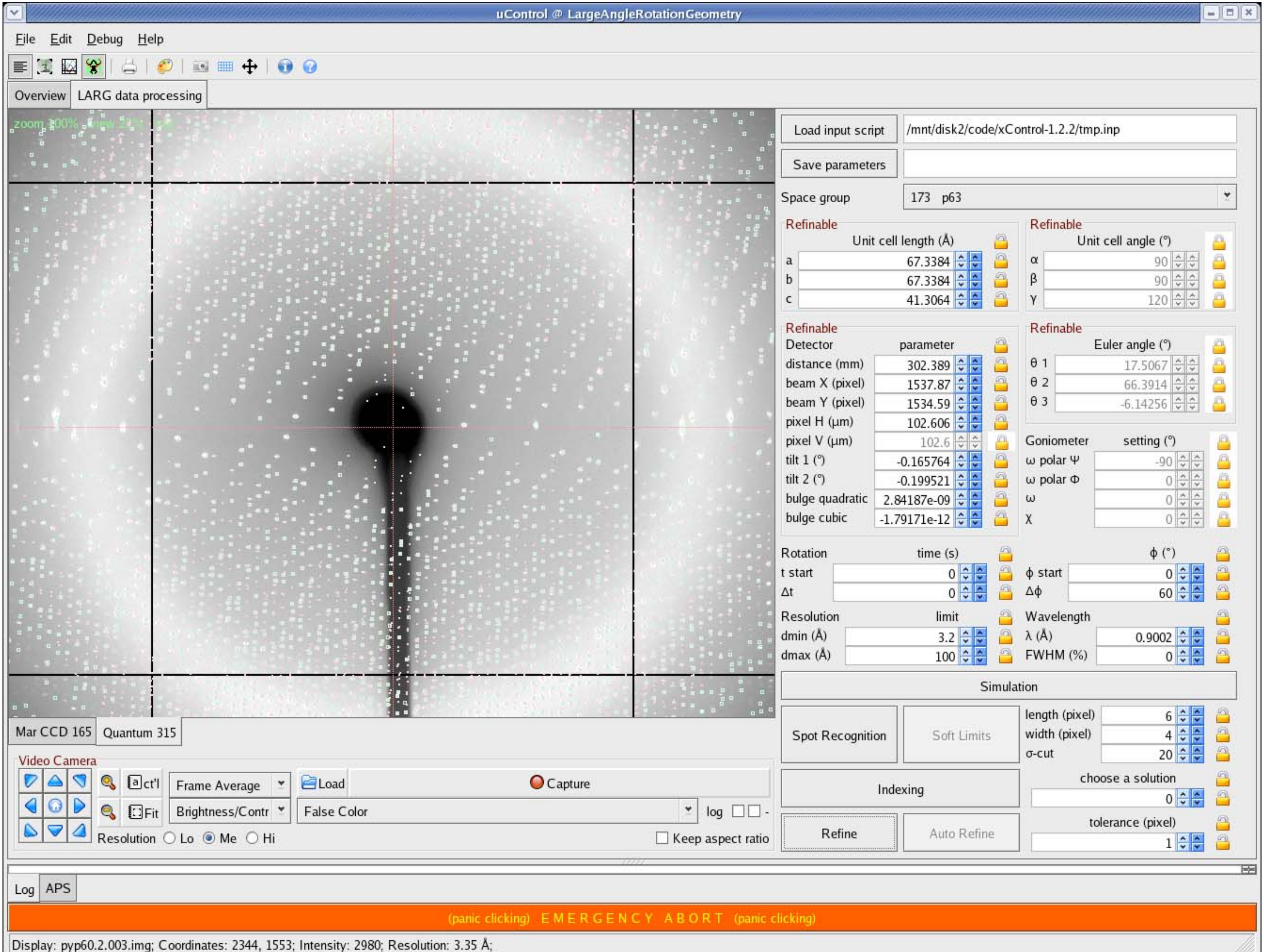
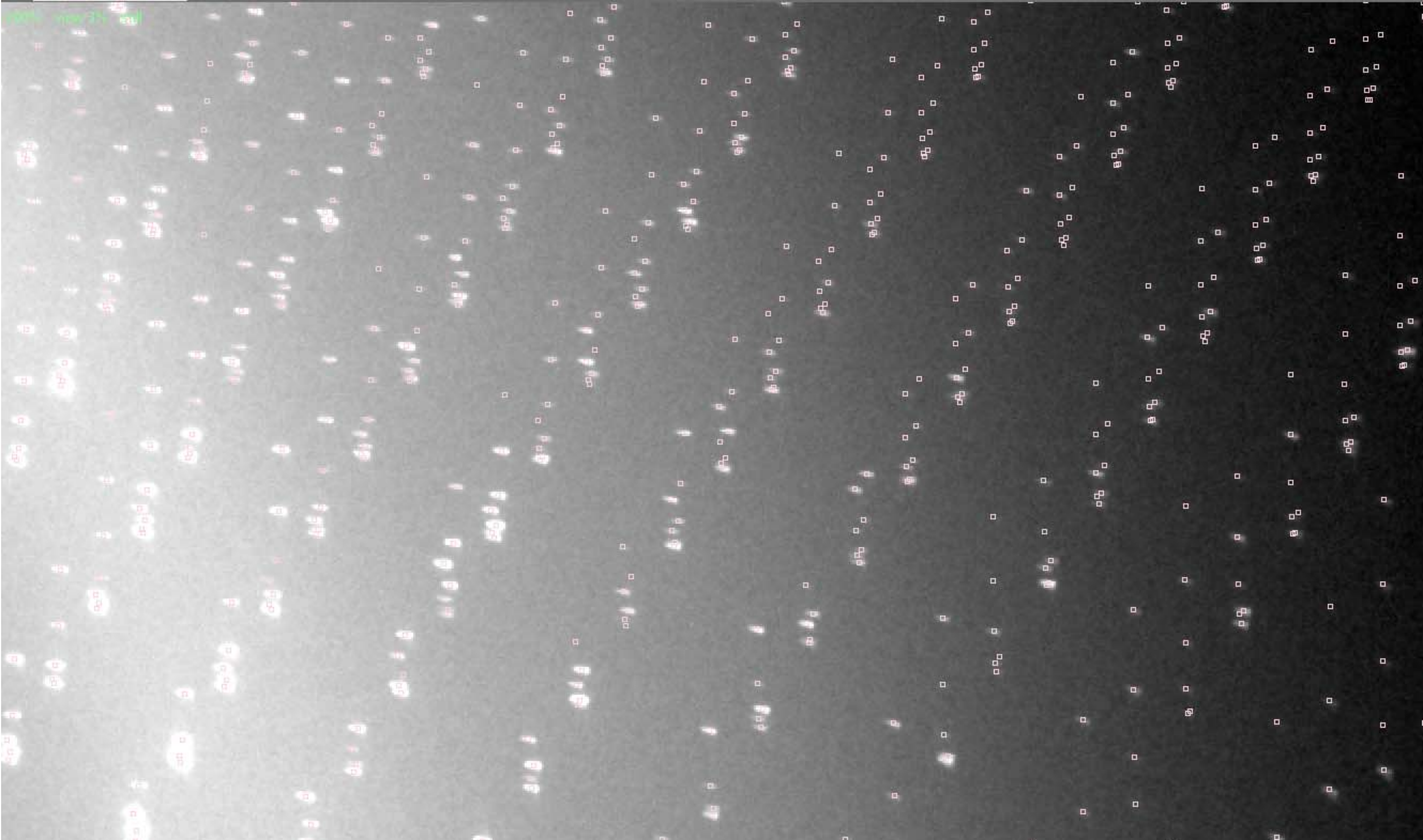


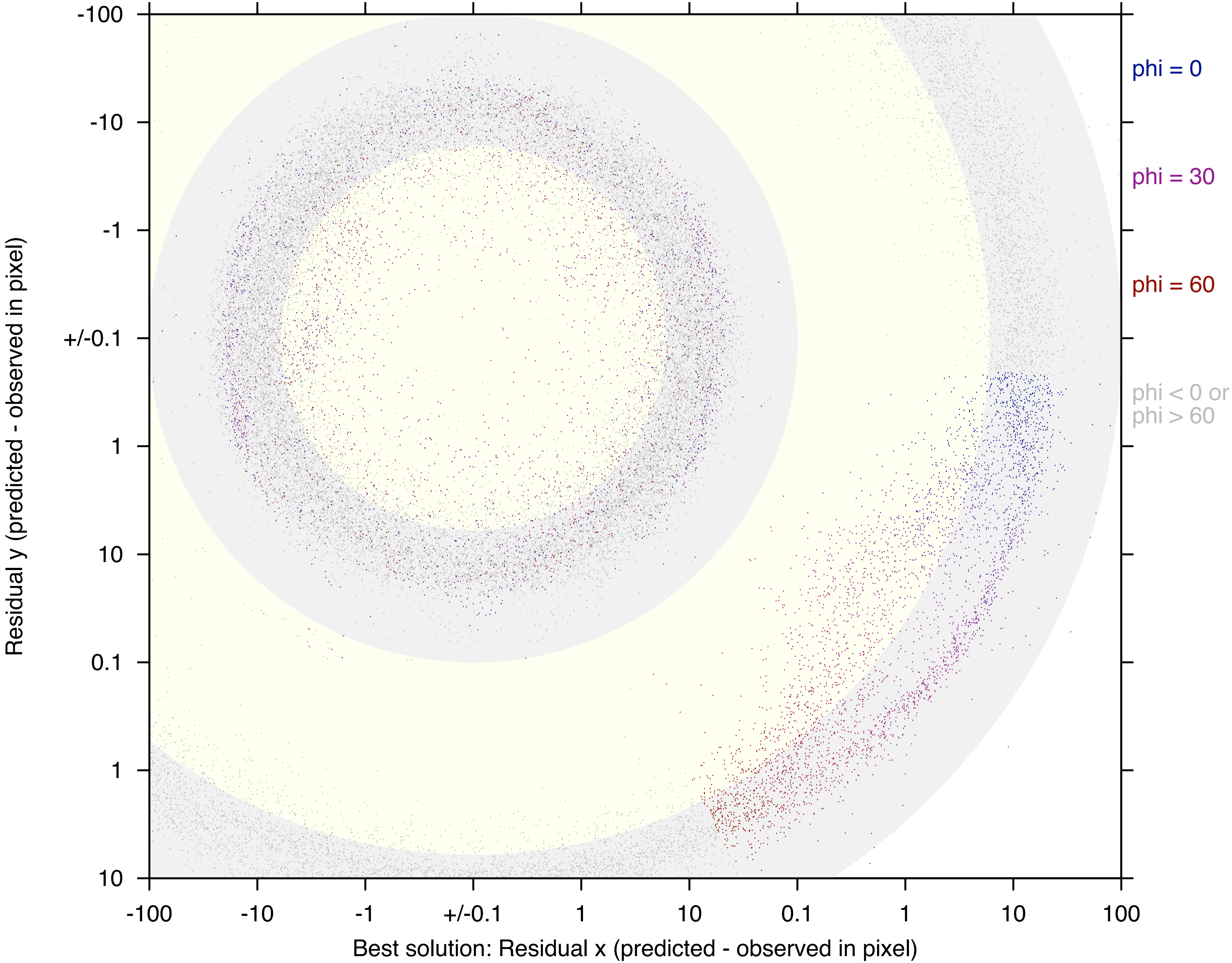
Figure 2.3.2.2.2.d Four-dimensional data distribution of four simulated LARG experiments. Axes as in Fig. 2.3.2.2.2.c. The four LARG exposures are obtained with different angular speeds of rotation. The red and cyan dots represent two different reflections and their symmetry-related observations.

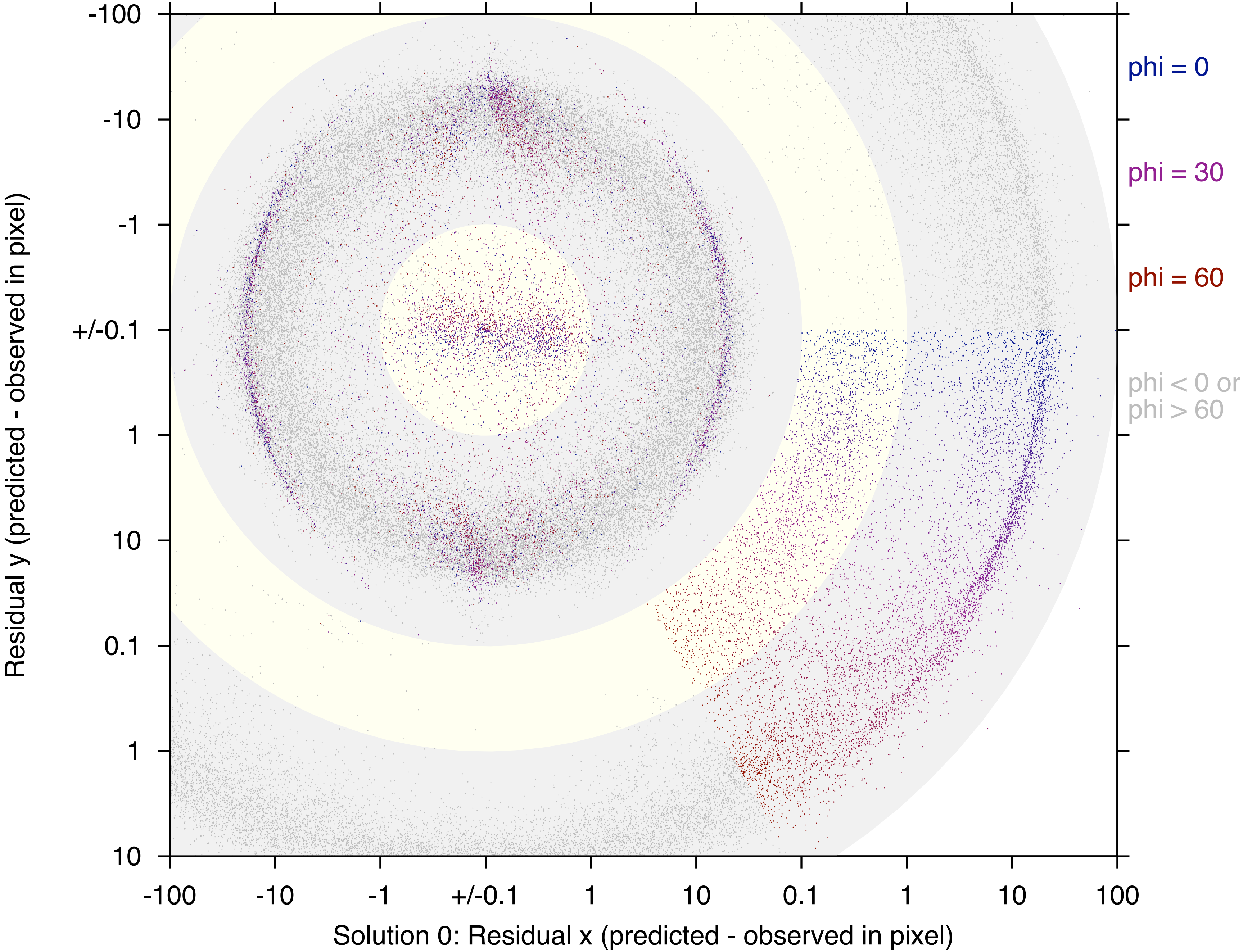
Laue and LARG comparison

	Laue	LARG
Source	polychromatic	monochromatic
Crystal motion	motionless	large-angle rotation
Time resolution	ultrafast (~150 ps and > 150 ns)	fast (ms)
Time point	discrete, programmable	continuous, random
Background	high	high
Detector	integrating	integrating, photon counting
Suitable system	reversible	irreversible


uControl @ LargeAngleRotationGeometry
File Edit Debug Help
Overview
LARG data processing
Load input script
/mnt/disk2/code/xControl-1.2.2/tmp.inp
Save parameters
Space group
173 p63
Refinable
Unit cell length (Å)
a 67.3384
b 67.3384
c 41.3064
Refinable
Unit cell angle (°)
α 90
β 90
γ 120
Refinable
Detector
parameter
distance (mm) 302.389
beam X (pixel) 1537.87
beam Y (pixel) 1534.59
pixel H (µm) 102.606
pixel V (µm) 102.6
tilt 1 (°) -0.165764
tilt 2 (°) -0.199521
bulge quadratic 2.84187e-09
bulge cubic -1.79171e-12
Refinable
Euler angle (°)
θ 1 17.5067
θ 2 66.3914
θ 3 -6.14256
Goniometer
setting (°)
ω polar Ψ -90
ω polar Φ 0
ω 0
χ 0
Rotation
time (s)
t start 0
Δt 0
ϕ (°)
ϕ start 0
Δϕ 60
Resolution
limit
dmin (Å) 3.2
dmax (Å) 100
Wavelength
λ (Å) 0.9002
FWHM (%) 0
Simulation
Spot Recognition
Soft Limits
length (pixel) 6
width (pixel) 4
σ-cut 20
Indexing
choose a solution
0
Refine
Auto Refine
tolerance (pixel)
1
Mar CCD 165
Quantum 315
Video Camera
ct'l
Fit
Frame Average
Load
Capture
Brightness/Contr
False Color
log
Resolution
Lo
Me
Hi
Keep aspect ratio
Log
APS
(panic clicking) EMERGENCY ABORT (panic clicking)
Display: pyp60.2.003.img; Coordinates: 2344, 1553; Intensity: 2980; Resolution: 3.35 Å;




phi = 0
phi = 30
phi = 60
phi < 0 or
phi > 60
Residual y (predicted - observed in pixel)
-100
-10
-1
+/-0.1
1
10
0.1
1
10
-100
-10
-1
+/-0.1
1
10
0.1
1
10
100
Best solution: Residual x (predicted - observed in pixel)


phi = 0
phi = 30
phi = 60
phi < 0 or
phi > 60
Residual y (predicted - observed in pixel)
-100
-10
-1
+/-0.1
1
10
0.1
1
10
-100
-10
-1
+/-0.1
1
10
0.1
1
10
100
Solution 0: Residual x (predicted - observed in pixel)